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BRAIN ABSCESS ASSOCIATED WITH CONGENITAL HEART DISEASE

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Brain abscess is a relatively unrecognized complication of congenital heart disease, so little known that only rarely does one find reference to it in any standard textbook of medicine. This obscurity must result in part at least from the scarcity of published cases, only 23 having been reported to date. Hanna [1] in 1941 collected 17 cases from the literature and added 6 more of his own. Of this entire series, in only 3 cases, 1 reported by Ingham [2] and 2 reported by Wechsler and Kaplan,[3] was the disease correctly diagnosed prior to death and surgical drainage of the abscess attempted. In all probability, this apparent obscurity and paucity of reported cases represent the failure either of their recognition or of their publication rather than the rarity of their occurrence.

Three cases of brain abscess associated with septal defects of the heart coming to autopsy at the Mallory Institute of Pathology of the Boston City Hospital during the years 1936 to 1943 form the body of this paper. These cases were selected from the 7,880 autopsies performed during these years, which included 53 cases of congenital heart disease of all types. There were no other instances of encephalomalacia encountered in the latter group—a point of some interest in relation to the discussion which follows.

REPORT OF CASES

CASE 1.—A 10 year old girl was admitted to the Boston City Hospital with a history of listlessness and extreme lethargy for seven days. A persistent cough appeared three to four days later, followed by intractable vomiting. She became increasingly drowsy up to the day of her admission to the hospital, on which day she complained of a severe headache with pain in the left ear. There were no signs of fever, stiff neck or localizing neurologic symptoms up to the time of her admission. Her past history revealed that from birth she had been recognized as having congenital heart disease and had always been restricted to a regimen of limited activity. No history of cyanosis could be elicited. Of some passing interest is the fact that the patient was a 3½ pound (1,560 Gm.) premature infant at birth.

Physical Examination.—On admission she appeared to be a well developed, dehydrated, restless child having intermittent, spastic, purposeless movements of the arms. Soon after admission she became disoriented and lapsed into coma. Her temperature was 103 F., pulse rate 120 and respiratory rate 26. Cyanosis was moderate but definite. There was a well defined clubbing of the fingers. The heart was not enlarged. The pulse rate was extremely rapid. A machinery murmur, most noticeable in the apical region, was heard over the entire precordium. Nuchal rigidity and positive Kernig and Brudzinski reflexes were prominent. There were no other neurologic signs.

Laboratory Studies.—There was time for only a lumbar puncture, which gave the following results: The fluid was purulent, with 34,000 cells per cubic millimeter, mostly polymorphonuclear leukocytes, and the sugar was diminished. Smear of the spinal fluid demonstrated many gram-positive cocci in pairs and short clusters. Culture of the spinal fluid revealed a streptococcus with alpha hemolysis. Culture of the blood at this time showed no growth.

Course.—Treatment was started with 2.5 Gm. of sulfadiazine, intravenously injected, and fluids administered parenterally, but the coma deepened; the temperature rose to 104 F.; breathing became gasping, and the patient died with a terminal convulsion seven hours after her admission.

Postmortem Examination.—The only significant observations pertained to the heart and brain. The heart weighed 150 Gm. (approximately the usual size and weight) and presented the classic tetralogy of Fallot, with (*a*) dextroposition of the aorta, (*b*) pulmonary stenosis, with a valvular lumen 0.3 cm. in diameter, (*c*) cor pulmonale, the right ventricle measuring 1.2 cm. in thickness as compared with the thickness of the left ventricle of 1 cm. and (*d*) an oval defect, 1 by 1.2 cm., lying in the tendinous portion of the interventricular septum. The brain weighed 1,300 Gm. (slightly increased in size and weight). The surfaces were flattened, with increased vascularity of the leptomeninges and narrowing of the sulci. There was a definite edema of the left cerebral hemisphere, with widening of this hemisphere. The base of the brain was covered with a layer of yellow-white purulent exudate. In the left parieto-occipital lobe there was

From the Mallory Institute of Pathology of the Boston City Hospital.

1. Hanna, R.: Cerebral Abscess and Paradoxic Embolism Associated with Congenital Heart Disease: Report of Seven Cases with Review of Literature, Am. J. Dis. Child. **62**:555 (Sept.) 1941.

2. Ingham, D. W.: Paradoxical Embolism, Am. J. M. Sc. **196**:201 (Aug.) 1938.

3. Wechsler, I. S., and Kaplan, A.: Cerebral Abscess (Paradoxic) Accompanying Congenital Heart Disease, Arch. Int. Med. **66**:1282 (Dec.) 1940.

an abscess cavity, thin walled, up to 2 cm. in diameter, occupying chiefly the central and convolutional white matter. At one point the abscess had extended to within 0.2 cm. of the pia, and at another point it had ruptured into the posterior horn of the left lateral ventricle, producing a ventricular empyema and meningitis.

Microscopically, the abscess cavity was filled with purulent exudate containing many demonstrable cocci. The abscess wall was thin and poorly formed, showing active proliferation of fibroblasts. No thrombosed arteries or veins were demonstrable, nor were there any other lesions in the brain suggestive of infarcts or hemorrhages.

The remainder of the observations made at autopsy were not pertinent to the present discussion save for the fact that no foci of infection were found anywhere else in the body, including extremities, lungs, middle ears and sinuses.

Bacteriologic Studies.—There was no growth in the cultures of the heart blood or the lungs. The culture of the brain abscess grew streptococci with alpha hemolysis, fusiform bacilli and Borrelia vincenti. The culture of the spinal fluid grew streptococci with alpha hemolysis.

Comment.—This case is typical of the disease complex in one of its more confusing manifestations—a brain abscess containing, besides streptococci with alpha hemolysis, obvious oral organisms, namely, fusiform bacilli and spirochetes. No path of extension from the oral cavity to the brain or recognizable origin for infected emboli could be found.

Case 2.—A 19 year old married Negro woman entered the hospital on Aug. 28, 1936 for the fourth time.

In 1926, at 9 years of age, she injured her right hip by a fall and was subsequently hospitalized for drainage of the hip joint. An uneventful recovery followed, with no further symptoms referable to this infection.

Approximately three to four years later in a routine examination her mother was found to have a positive Wassermann reaction, and the patient was likewise found to have a positive reaction. She received regular and adequate antisyphilitic treatment until she was discharged as cured.

In 1934 she again entered the hospital, because of failing vision. A diagnosis of bilateral cataracts was made, and at the same time the patient was found to be diabetic. The diabetes was controlled by diet and insulin.

Her fourth, and final, admission was initiated by an incidental acute glossitis. At this time she admitted that she had stopped taking insulin several months before reentering the hospital.

In the course of the physical examination at the time of her admission, intense cyanosis of the mucous membranes with clubbing of the finger tips and toes was noted. Questioning revealed that the patient had noted these changes since childhood, but she disclaimed any knowledge of heart disease or any symptoms referable to cardiac decompensation.

Physical Examination.—She was an alert, well oriented, cooperative Negro woman with the obvious cyanosis and clubbing of finger tips and toes already mentioned. The margins of the gums were retracted, and the tongue was edematous and covered with irregular patches of exudate. The heart was not enlarged. The pulse rate was 120 with regular rhythm, and a continuous machinery murmur was heard best over the second left interspace. The abdomen was normal. Deep tendon reflexes of the lower extremities were absent, but sensation, pain and vibratory sense were within normal limits.

Laboratory Studies.—The only important results of laboratory tests were a hemoglobin content of 122 to 128 per cent, a red blood cell count of 7,000,000 cells per cubic millimeter and a white blood cell count of 5,100 cells per cubic millimeter. The blood sugar was 308 mg. per hundred cubic centimeters, and the urine was essentially normal save for a 4 plus reduction of sugar with traces of acetone. Culture of material from the mouth demonstrated pneumococci, Streptococcus viridans, Micrococcus catarrhalis and Staphylococcus aureus.

Course.—With a diet supplemented by 40 units of regular insulin daily the hyperglycemia and glycosuria were gradually controlled. The patient was well and ambulatory by the fifth day in the hospital but was asked to remain in order that her diabetes might be more carefully controlled. On the fifteenth day, after she had received 50 units of insulin, clonic convulsive movements of the left arm suddenly developed. Since it was assumed that the neurologic seizure was a reaction to the insulin, the dose was lowered to 40 units the next day but was again followed by a similar incident, with a residual weakness of her left arm and leg and a slight weakness of the left facial muscles. On the following day, the hemiplegia was well defined. Lumbar puncture at this time yielded a slightly cloudy fluid under no elevation in pressure, having 250 white blood cells per cubic millimeter, 92 per cent of which were polymorphonuclear leukocytes. Twenty-four hours later there was noticeable stiffness of the neck. The spinal fluid was now grossly cloudy, containing 3,000 white blood cells per cubic millimeter with 87 per cent polymorphonuclear leukocytes. On the next day, the third since the onset of signs referable to the central nervous system, the patient was in deep coma with obvious fulminating meningitis and hemiplegia of the left side. Culture of the cerebrospinal fluid now revealed an atypical streptococcus with alpha to gamma hemolysis. Cultures of the blood repeatedly showed no growth. The rapidly progressive course of the disease could not be altered by any therapy, and she died nineteen days after her admission and four days following the onset of the signs and symptoms referable to the central nervous system.

Postmortem Examination.—The only observations of significance were in the mouth, heart and brain. The gums were soft, necrotic and spongy, showing areas of frank exudation. The heart weighed 280 Gm. and was slightly increased in weight but extremely increased in size. There were multiple congenital defects present which constituted the tetralogy of Fallot, together with a patent ductus arteriosus. The right ventricle measured 1.8 cm. in thickness, in contrast to the thickness (1.5 cm.) of the left ventricle. In the interventricular septum there was found a circular defect, 2 cm. in diameter, lying just below the aortic valve ring. The pulmonary valve orifice was completely occluded by a membranous septum. The ductus arteriosus was widely patent, with a diameter of 1.5 cm. The aorta was dextroposed and was so situated as to receive blood from both the right and the left ventricle.

The brain weighed 1,080 Gm., was decreased in size and weight and showed evidence of considerable cerebral edema as demonstrated by the flattening of convolutions and obliteration of the sulci. The subarachnoid space contained yellow-green purulent exu-

date, distributed most heavily over the left cerebral hemisphere and base of the brain. Within the right parieto-occipital lobe there was a roughly spherical cavity, 4 cm. in diameter, filled with thick purulent exudate, communicating with the lateral ventricle (fig. 1).

The remaining organs were essentially normal save for slight bilateral bronchopneumonia of the lower lobes of the lungs.

Microscopically, sections of the right hemisphere evidenced a fairly recent brain abscess, showing slight fibrosis of the wall. The meninges contained a sparse infiltration of lymphocytes and polymorphonuclear leukocytes. No organisms were seen in the tissue sections of the brain abscess and meninges. The gross diagnosis of bronchopneumonia was confirmed by characteristic microscopic appearance of focal areas of infiltration with polymorphonuclear leukocytes about widely scattered bronchioles.

Bacteriologic culture of the meninges showed no growth, perhaps because of the sulfonamide medication.

Comment.—This case presented an unusual opportunity for following the course of the disease from its very inception. The patient was in the hospital for incidental reasons when localizing neurologic signs sud-

Fig. 1.—The abscess is represented by the irregularly shaped cavity just above and communicating with the posterior horn of the lateral ventricle. The central white matter above and lateral to the cavity is softened and hemorrhagic. The apparent enlargement of the right parietal lobe is due to cerebral edema.

denly developed. The suddenness and the dramatic character of the onset led clinical observers to the conclusion either that a cerebral embolism, primary site unknown, had developed or that there was an intravascular thrombosis, initiated by the marked polycythemia. The time relation of the first convulsive seizures with the administration of the insulin raised some question as to the role that the insulin might have played in the cause of this symptom complex.

The possibility of the development of a brain abscess was apparently not considered, although many features of this case were suggestive of this complication. Although no obvious focus for the origin of septic emboli could be found either clinically or pathologically, the infections of the mouth and lungs must both be considered as possible points of origin.

This case is characteristic of others in this group, illustrating the unheralded development of a cerebral abscess in a patient with the tetralogy of Fallot, with rupture of the abscess into a ventricle producing fatal meningitis.

CASE 3.—A 20 year old white woman was transferred to the Boston City Hospital on July 20, 1942 from a neighboring hospital, with the diagnosis of acute purulent meningitis and congenital heart disease.

The patient was known to have had "heart trouble" since birth, with blueness of the extremities and dyspnea on slight exertion.

At 10 years of age she had several bouts of "growing pains," but the condition was considered too minor to warrant medical consultation.

In 1934, two years later, the pain in the legs recurred, and she was admitted to the Massachusetts General Hospital, where a diagnosis was made of rheumatic heart disease superimposed on a congenital defect of the heart.

With the exception of her severe exertional dyspnea, she was asymptomatic for the next six years.

Two weeks before her last admission to the hospital, on July 20, she began to experience intermittent headaches, which became constant four days before she entered the hospital. These headaches were accompanied by nausea and projectile vomiting. Although she believed that she had had some fever, she said that she had not experienced any chills. She complained of a slight stiffness of the neck and appeared to show intermittent periods of mental confusion.

Physical Examination.—On her admission she appeared restless, somewhat irrational and acutely ill. There was a definite cyanosis of the face, lips and nail beds. There was slight injection of the ear drums bilaterally, and the neck was rigid.

The heart rate was 104 with a gallop rhythm heard over the entire precordium. The pulmonic second sound was loud and snapping, with a soft, blowing systolic murmur heard along the left border of the sternum. This murmur was heard loudest at the third left interspace. The spine was hyperextended, with active resistance to attempted flexion. Kernig reflexes were elicited bilaterally. The ankle and abdominal reflexes were absent. The fingers and toes showed pronounced clubbing.

Laboratory Studies.—In the time available only a lumbar puncture was performed, which yielded cloudy fluid under increased pressure, containing 330 mg. of protein per hundred cubic centimeters and many white cells, chiefly polymorphonuclear leukocytes. No bacteria were seen.

Course.—Her temperature continued to rise (to 105 F.) despite the administration of sulfonamide compounds. Nine hours after her admission, although no growth from the culture of the spinal fluid had been obtained, an empiric intrathecal injection of antimeningococcus serum was given. She was transferred to the Boston City Hospital, department of contagious diseases, where the results of physical examination were identical with those just given, except that the blood pressure now was 60 systolic and 0 diastolic, with a weak thready, rapid pulse. Despite all supportive measures, she died within twenty-four hours.

It may be noted that at no time did a culture of the spinal fluid reveal organisms, although on one occasion gram-negative cocci were reported to have been seen on smear.

Postmortem Examination.—Again the only pertinent observations related to the heart and brain. The heart weighed 350 Gm. (slightly increased in size and weight). It presented, again, the complex of congenital cardiac malformations known as the tetralogy of Fallot, with a defect 1 by 1.5 cm. of the interventricular septum, dextroposition of the aorta, pulmonary stenosis and cor pulmonale.

The ductus arteriosus was, likewise, widely patent. The right ventricle measured 1 cm. in thickness, exactly equal to the thickness of the left ventricle.

The brain was also similar in many respects to those previously described and weighed 1,440 Gm. It was

intensely congested, showing over its sulci and vascular channels a yellow-green purulent exudate. Coronal section revealed a small abscess cavity 1 cm. in transverse diameter and 5 cm. in length in the left superior temporal convolution. It contained a thick green exudate enclosed in a firm, apparently fibrous wall. At one point in its extent, the abscess could be seen to communicate with the lateral ventricle and subarachnoid space (fig. 2). All ventricular cavities were filled with thick purulent exudate.

Microscopic examination of the brain sections demonstrated the abscess wall to be well formed, containing many fibroblasts and newly formed blood vessels. An inflammatory tract could be seen to communicate with the lateral ventricle.

Bacteriologic Study.—A smear of the exudate, while demonstrating many white blood cells, showed no organisms, and the culture of the meninges revealed no growth.

Comment.—Case 3 bears a great similarity to case 1 in that in a patient with known congenital heart disease signs of meningitis insidiously developed, which rapidly led to her death. As in the previous cases, the underlying brain abscess was completely unsuspected.

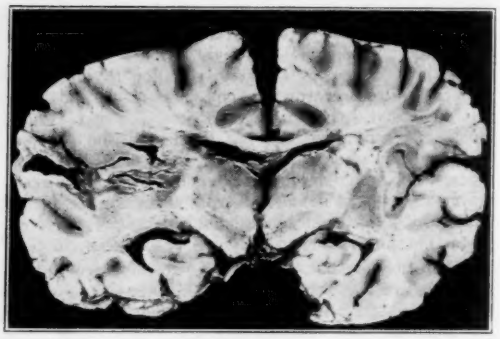

Fig. 2.—The abscess cavity is situated in the left superior temporal gyrus and extends from the cortex almost to the body of the lateral ventricle in this plane of section.

COMMENT

It is interesting to note that in cases 1 and 3 the fatal illnesses were initiated by the insidious development of lethargy, drowsiness and headaches, progressing to signs of frank meningitis and death.

Case 2, on the contrary, was somewhat more confused by the simultaneous occurrence of diabetes and other incidental conditions. In this patient the fatal illness was initiated by sudden seizures resembling reactions to insulin, and it was not until the day before her death that meningitis became clinically apparent.

Despite the fact that in 2 cases the terminal illnesses developed insidiously and in the third somewhat acutely, all 3 cases present good illustrations of this not rare complication of congenital heart disease, namely, the development of a brain abscess. The unexpected onset in a patient with congenital heart disease of signs and symptoms pointing to a lesion of the central nervous system of a focal type, which when untreated leads to frank meningitis and sudden death, may well indicate the development of a cerebral abscess with subsequent rupture of it into the cerebral ventricular system.

While practically all varieties of septal defects have been associated with brain abscess, the complex known as the tetralogy of Fallot is by far the most common, occurring in 10 out of 21 cases of this complication reported in the literature and in all 3 cases of this report, a total of 13 out of 24 cases.

In the cases reported here two abscesses occurred in the left side and one in the right side.

The precise pathogenesis of brain abscesses occurring in patients with septal defects of the heart is not known. However, many closely related facts which throw considerable light on the problem are known. I may begin by stating that there are only three known mechanisms by which brain abscesses may develop in any patient: (*a*) direct extension from some neighboring infection, such as mastoiditis or osteomyelitis of the skull, (*b*) retrograde thrombophlebitis from some source similar to that just mentioned and (*c*) some embolic phenomenon from a distant focus of infection. In the preceding cases it was definitely established that the brain abscesses could not have arisen from nearby infections; hence, it must be assumed that they were embolic in origin.

Contrary to common belief, it is now well established by the work of Malinovski [4] and others that simple implantation of organisms into the brains of animals, either by infection of the blood stream or by direct inoculation, will not give rise to brain abscess. Equally well known is the fact that in animals abscess can be produced in the brain only by prior injury to the brain substance or its vascular supply followed by the implantation of organisms into the site of injury. Many technics have been used by Markley,[5] Graff,[6] Thomas,[7] Falconer, McFarlan and Russell [8] and others to produce these abscesses, all of them based on the

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theory just mentioned. The agents used to produce the initial injury vary from trauma by mechanical injury to injection of sodium ricinoleate and infected agar mass, the latter agent acting both as a foreign body and as an infectious agent. The injury has been either accompanied with or followed by the direct implantation of organisms into the traumatized site. It therefore seems reasonable to assume that brain abscesses associated with septal defects of the heart must simply be a result of paradoxic emboli in the brain, either sterile or infected, producing an area of lowered resistance, followed in the former instance by bacteremia which infects the area of encephalomalacia. In this regard, it is to be hoped that study of a larger number of these cases may reveal instances of encephalomalacia prior to their infection by organisms spread through the blood stream. This hypothesis has previously been discussed by Hanna and others. Unfortunately, it has been difficult in our cases here and in most of the previously reported cases to demonstrate adequate origins for the emboli and the transient bacteremia. However, this failure does not necessarily completely invalidate this working hypothesis, because it may be impossible in certain cases to demonstrate origins for many embolic phenomena, and the bacteremia may have been so transient as to have passed entirely undetected.

In the hope that it might be possible to substantiate experimentally the assumption that abscess results from embolism followed by infection, experiments were carried out on rabbits in which attempts were made to produce focal areas of encephalomalacia by injection of particulate matter into the internal carotid artery, followed in ten to twelve days by the intravenous injection of virulent cultures of organisms. These experiments were similar to the work of Graff. Inasmuch as great difficulty was encountered in the production of small focal infarcts with these technics, I attempted to traumatize the brain substance directly by instrumentation and injection of sodium ricinoleate and followed this at an interval by the intravenous injection of virulent cultures of Staph. aureus, Escherichia coli and alpha hemolytic streptococci. These attempts, as were those of Stewart cited by Graff,[6] were all unsuccessful, inasmuch as the areas of injury did not become abscessed. However, the number of experiments performed was too few to permit final decision, and more work is in progress along this line.

SUMMARY AND CONCLUSION

Three cases of cerebral abscess complicating congenital septal defects of the heart are added to those previously reported, bringing the total number of such cases in the literature to date to 26. In only 3 cases in the literature has an antemortem diagnosis of the disease been made and surgical drainage been instituted, a proportion which reflects principally the difficulty in diagnosis, arising in most instances from unfamiliarity with this complication of septal defects of the heart.

With the increased number of cases reported, it is to be hoped that in patients with congenital heart disease, especially in those having the tetralogy of Fallot, underlying brain abscess will be considered in the diagnosis of any focal neurologic damage or meningitis. Certainly only early recognition will permit successful surgical intervention and hope for cure of this uncommon syndrome.

INFLUENCE OF ANOXEMIA ON THE HEMOPOIETIC ACTIVITY

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The exact mechanism responsible for the maintenance of an equilibrium between production and destruction of blood in the normal person is not entirely understood, but it has become an almost established fact that the oxygen tension of the circulating blood is an important factor in regulating the degree of hemopoietic activity. Since the observations of Bert [1] and Viault,[2] in the second half of the last century, it has been customary to refer to the polycythemia found in human beings and animals exposed to a low barometric pressure as the classic example of the stimulating effect of oxygen deficiency on erythrocyte and hemoglobin formation. The demonstration by Barcroft and his collaborators [3] in 1923 that newcomers and residents at high altitudes exhibit a lowering of the arterial oxygen saturation as a consequence of the low oxygen tension in the inspired air opened a most fertile field for the accurate study of the relationship between the degree of anoxia and the hematologic response. However, the opportunity has not been adequately appreciated, and most studies at high altitudes have been carried out on human beings or animals subjected for only a few hours or days to such an abnormal environment. It does not need to be emphasized that the results obtained in these observations, as well as in short time experiments in chambers where the pressure or the percentage of oxygen is artificially lowered, do not reveal the true nature of the processes of hematologic adaptation to a constant or intermittent deficiency in the oxygen supply to the tissues. The degree as well as the duration and constancy of the anoxic stimulus must play a part in influencing the hemopoietic activity. A more ample knowledge of these modifying factors not only may be of value from the point of view of exposure to a low barometric pressure but may help to clarify some related clinical problems. Anoxia in one form or another is an alteration common to many pulmonary, circulatory, hematic and other diseases in which the blood findings have been partially or totally related to the disturbance in respiratory function. In other processes, like polycythemia vera, anoxia is suspected to have an important etiologic role, but conclusive proof has not been afforded yet.

The investigations to be reported in this paper concern the morphologic and other characteristics of the circulating blood under the influence of temporary, intermittent and chronic anoxic anoxia (anoxemia). Most of the work has been carried out at high altitudes (chart 1), and the results obtained have been compared with those observed in the study of healthy subjects at sea level and in previous related investigations. No attempt has been made to cover all the vast literature accumulated in this field, which has been recently summarized by Van Liere.[4]

METHODS

All determinations have been made on blood taken in the morning while the subject was fasting or after he had eaten a light breakfast. Fifteen to twenty cubic centimeters of blood was obtained from a venous puncture, care being taken to release the tourniquet as soon as the needle was introduced, and 5 cc. was transferred to a small bottle containing 10 mg. of potassium oxalate in powder. The rest of the blood was utilized for the determination of the serum bilirubin in its total amount [5] or in fractionated forms; for the latter investigation the Malloy-Evelyn photoelectric colorimetric procedure [6] was used, and the serum proteins by means of the refractometric index and the Kagan falling drop method.[7] On the 5 cc. of oxalated blood the following determinations were made: (*a*) number of erythrocytes and of leukocytes per cubic millimeter, a double Neubauer counting chamber being used, and the average of two counts taken as the final result; (*b*) number of reticulocytes (per hundred red cells and per cubic

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millimeter), brilliant cresyl blue being used as stain; (*c*) the hematocrit value, measured with a Wintrobe tube which was centrifuged for forty-five to sixty minutes at about 3,000 revolutions per minute in an International size 1 type SB centrifuge (the hematocrit figure was multiplied by 1.09 to correct for the shrinkage due to the anticoagulant used), and (*d*) hemoglobin (grams per hundred cubic centimeters), estimated either by measuring the oxygen-combining capacity of the blood in the Van Slyke manometric apparatus [8] or by employing the Evelyn photoelectric colorimeter previously calibrated with the former method. Capillary blood was obtained immediately after the venous puncture, from a finger or the lobe of the ear. From this sample a smear was made and stained with Wright's stain; this was used for the determination of the differential leukocyte count, according to Schilling's scheme,[9] and for the measurement of the diameter (microns) of the red cells. For the latter determination a calibrated ocular micrometer was used. A total of 300 cells were measured in each case, with the exception of some studied at the highest altitude, in which only 100 to 200 cells were measured, owing to the difficulties of obtaining an adequate smear. For a few subjects these investigations were made on the sample of venous blood. The mean corpuscular volume (cubic microns), the mean corpuscular hemoglobin (micromicrograms) and the mean corpuscular hemoglobin concentration (per cent) were calculated according to Wintrobe's formulas.[10] The mean corpuscular thickness (microns) was calculated, indirectly, by means of the formula

$$\frac{\text{mean corpuscular volume}}{3.1416 \times \left(\frac{D}{2}\right)^2}$$

in which D equals the mean corpuscular diameter. The mean corpuscular surface area (square microns) was calculated from the formula

$$\left(\frac{V}{T} \times 2\right) + \left(3.1416 \times T \times D\right)$$

in which V represents the mean corpuscular volume, T the mean corpuscular thickness and D the mean corpuscular diameter. The spherocytic index was derived from Heilmeyer's [11] formula, $\frac{\text{mean corpuscular thickness.}}{\text{mean corpuscular diameter}}$

The fragility of the red cells to hypotonic solutions of sodium chloride was studied in some subjects by the method of Giffin and Sanford.[12] At high altitudes the viscosity of the blood was determined by means of a Hess viscosimeter and distilled water for comparison. In a few cases the gastric acid content was investigated, 50 cc. of a 7 per cent alcoholic solution orally administered and a subcutaneous injection of 0.5 cc. of histamine phosphate solution being used as stimulants for the gastric mucosa.

The total circulating blood volume was determined in most cases by the method of Keith, Rowntree and Geraghty,[13] with the modifications of Hooper, Smith, Belt and Whipple.[14] A 1.5 per cent solution of brilliant vital red was employed. In a smaller group of cases, at sea level and at high altitudes, Evans blue was employed in the determination, according to the technic of Gibson and Evans.[15] Five samples of blood were obtained at known intervals after the injection, and the concentration of the dye was observed by means of the Evelyn photoelectric colorimeter, with use of the microabsorption cell described by Evelyn and Gibson.[16] The carbon dioxide content and the oxygen saturation of the arterial blood were determined in blood obtained, with the usual precautions to avoid exposure to the air, from the radial artery. The blood was kept under mercury and at a low temperature when several hours elapsed before the analysis. The determinations were carried out according to the method of Van Slyke and Neill [8] in their manometric apparatus.

NORMAL HEMATOLOGIC VALUES AT SEA LEVEL

A considerable number of observations made on healthy male adults in different parts of the world, which have been reviewed by several investigators [17] and are summarized in table 1, have demonstrated that there are not definite geographic or racial differences at sea level in regard to the number of red cells and the amount of hemoglobin in the circulating blood, a fact pointed out by Wintrobe [17a] some years ago. We have made observations on 200 healthy men living in Lima, at sea level.[18] The results obtained for half of these subjects have already been published.[19] Most of the men studied were medical students, and a few belonged to the army; their ages varied between 19 and 45 years, with the large majority between 20 and 30 years. Ninety of the men had been born and raised at sea level, and 110 were natives of places located at altitudes over 2,000 meters (7,560 feet) and had lived at sea level for periods varying from six months to many years. In the analysis of the collected data no appreciable difference was found between the values for men born at sea level and

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18. Lima is located at an altitude of 150 meters (490 feet); from the point of view of the studies reported in this paper, this may be considered as sea level.

19. Hurtado, A.; Pons, M. J., and Merino, M. C.: La anemia de la enfermedad de Carrión, Lima, Peru, Librería e Imprenta Gil, 1938.

those for men born in high localities, provided that the latter had been living at sea level for a prolonged time. In consequence the findings for both groups are given together in table 2. On the other hand, men who had been born at high altitudes and had been living in Lima for only a few months frequently showed a moderate reduction in red cells and hemoglobin. For this reason obtained in different parts of the world, and the statistical variations are not higher than the ones ordinarily found in such determinations. The values corresponding to some of the morphologic characteristics of the circulating erythrocyte (mean corpuscular volume, hemoglobin and hemoglobin concentration) also agree closely with those observed by Wintrobe [20] and other

TABLE 1.—*Average Values for Red Blood Cells and Hemoglobin at Sea Level in Healthy Men. Summary of Literature Reviewed by Several Authors* [17]

Investigators	Place	Number of Subjects	Red Blood Cells, Millions per Cu. Mm.	Hemoglobin, Gm. per 100 Cc.
Goldhamer and Fritzell	U. S. A.	100	4.72	14.0
Walters	U. S. A.	100	4.84	15.1
Emerson	U. S. A.	171	5.44	16.1
Wintrobe and Miller	U. S. A.	100	5.85	17.0
Wintrobe	U. S. A.	86	5.45	16.0
Haden	U. S. A.	70	4.95	15.3
Osgood	U. S. A.	250	5.42	15.8
Osgood and others	U. S. A.	137	5.39	15.8
Foster and Johnson	U. S. A.	115	5.26	15.6
Isaacs and Fritzell	U. S. A.	57	5.08	15.2
Sachs, Levine and Fabian	U. S. A.	100	4.88	15.0
Nelson and Stoker	U. S. A.	350	5.11	15.0
Broun and Briggs	U. S. A.	23	5.29	16.6
Epstein	U. S. A.	42	4.93	....
Williamson	U. S. A.	140	....	17.0
Williamson	U. S. A.	81	....	15.8
Dill	U. S. A.	40	....	15.5
Price-Jones	U. S. A.	20	....	15.4
Haden	U. S. A.	20	....	15.2
Haden	U. S. A.	20	....	15.8
Myers and Eddy	U. S. A.	111	....	15.7
Rowntree and Brown	U. S. A.	49	....	16.4
Kaltreider, Hurtado and Brooks	U. S. A.	25	....	16.2
Fiddes and Whitney	Canada	20	5.52	15.6
Parodi	Argentina	50	5.50	15.4
Tenconi	Argentina	50	5.30	14.8
Orias	Argentina	82	....	15.3
Orias	Argentina	321	....	14.3
Orias	Argentina	307	....	14.6
Gargiulo	Argentina	51	....	15.6
Gargiulo	Argentina	944	....	15.2
Gargiulo	Argentina	128	....	17.0
Mecchéri	Argentina	227	....	15.4
Hurtado, Pons and Merino	Peru	100	5.26	15.7
Price-Jones, Vaughan and Goddard	England	100	5.50	14.5
Price-Jones and others	England	100	5.43	14.5
McGeorge	England	50	5.48	15.2
Jenkins and Don	England	116	....	15.8
Bing	Denmark	22	6.10	....
Beirring and Sorensen	Denmark	60	....	14.9
Jervell and Waaler	Norway	50	5.52	16.2
Linnbergh and others	Norway	51	5.27	....
Burgi	Switzerland	224	5.00	15.0
Millet and Balle-Helaers	Belgium	50	3.90	13.3
Jimenez Diaz	Spain	18	4.88	....
Alés	Spain	40	....	14.9
Komocki	Poland	33	5.84	....
Heilmeyer and Hansold	Germany	40	5.06	15.9
Horneffer	Germany	40	4.96	16.0
Napier and Das Gupta	India	50	5.36	14.8
Sokhei and others	India	121	5.11	15.4
Sankaran and Rajagopal	India	125	....	16.7
Napier and Nanjumdar	India	25	....	12.6
Chia Tu Tien	China	320	5.12	....
Hamre and Man Hing Au	Hawaii	137	5.08	15.1
Chamberlain	Philippine Islands	687	5.20	....
Navarro	Philippine Islands	104	....	14.1
Wardlaw and others	Australia	25	....	15.1
Wardlaw	Australia	26	....	15.8
Grand mean ± P. E.			5.23 ± 0.04	15.4 ± 0.07

the results obtained for 25 natives of high altitudes with a period of residence at sea level of less than a year have been separately summarized (table 3).

The mean values for number of red blood cells per cubic millimeter, grams of hemoglobin per hundred cubic centimeters and hematocrit level (percentage of red cells) in the blood of the series of 175 healthy men are similar to those investigators.[17c] There is some discrepancy in the results found by several workers in regard to the normal diameter of the red blood cells. The related literature has been summarized by Price-

20. Wintrobe, M. M.: Anemia: Classification and Treatment on Basis of Differences in Average Volume and Hemoglobin Content of Red Corpuscles, Arch. Int. Med. **54**:256 (Aug.) 1934.

Jones [21] and recently by Isaacs.[17c] The mean observed values vary between 7.20 and 7.90 microns (measured in dry stained preparations). Our mean value of 7.48 ± 0.01 microns falls within the accepted normal limits of variation. The coefficient of variation of 2.0 per cent indicates an extremely small fluctuation of the mean corpuscular diameter in healthy men. There are few observations in the literature concerning the thickness, surface area and spherocytic index of normal red blood cells. Price-Jones, Vaughan and Goddard,[22] following a technic similar to the one used by us, found a mean corpuscular thickness of 2.14 microns in 100 healthy men, and Dameshek and Singer [23] have indicated a normal range of 1.9 to 2.4 microns. Our mean value, of 2.09 ± 0.01 microns, relates closely to such findings, but it is higher than the low values reported by Emmons [24] and Gram,[25] who used entirely different methods in the measurement. Guest and Wing [26] and Bernstein and Chesluk [27] recently found average values of 139.7 and 135.5 square microns, respectively, for the mean corpuscular

TABLE 2.—*Observations on Blood of Healthy Men at Sea Level*

Determinations	Number of Subjects	Mean ± P. E.	St. Dev. ± P. E.	Coefficient of Variation (%)	Extreme Variations
Red blood cells (millions per cu. mm.)	175	5.14 ± 0.02	0.34 ± 0.01	6.6	4.32 - 5.90
Hematocrit (red cells, per cent)	175	46.8 ± 0.11	2.3 ± 0.08	4.9	41.0 - 52.7
Hemoglobin (grams per 100 cc.)	175	16.00 ± 0.04	0.80 ± 0.03	5.0	13.96 - 18.30
Corpuscular mean volume (cu. microns)	175	91.3 ± 0.23	4.5 ± 0.16	4.9	77.8 - 103.1
Corpuscular mean diameter (microns)	130	7.48 ± 0.01	0.15 ± 0.01	2.0	7.10 - 7.88
Corpuscular mean thickness (microns)	130	2.09 ± 0.01	0.12 ± 0.01	5.7	1.75 - 2.43
Corpuscular mean surface area (sq. microns)	130	136.7 ± 0.22	3.8 ± 0.14	2.8	126.3 - 145.3
Spherocytic index	130	0.28 ± 0.01	0.01 ± 0.004	3.6	0.23 - 0.33
Corpuscular mean hemoglobin (micromicrograms)	175	31.2 ± 0.09	1.9 ± 0.06	6.1	27.0 - 35.5
Corpuscular mean hemoglobin concentration (per cent)	175	34.1 ± 0.07	1.4 ± 0.05	4.1	30.9 - 37.4
Reticulocytes (per cent)	93	0.5 ± 0.02	0.3 ± 0.01	60.0	0 - 1.2
Reticulocytes (thousands per cu. mm.)	93	18.4 ± 0.94	13.4 ± 0.66	72.8	0 - 61.1
Red blood cell fragility:					
Initial hemolysis (per cent)	38	0.46 ± 0.001	0.01 ± 0.001	2.2	0.48 - 0.40
Total hemolysis (per cent)	38	0.38 ± 0.002	0.02 ± 0.001	5.2	0.40 - 0.34
Bilirubin, total * (mg. per 100 cc.)	93	0.76 ± 0.03	0.33 ± 0.02	43.4	0.41 - 1.61
Bilirubin, total † (mg. per 100 cc.)	92	0.72 ± 0.02	0.27 ± 0.01	37.5	0.26 - 1.44
Bilirubin, direct † (mg. per 100 cc.)	92	0.37 ± 0.01	0.15 ± 0.01	40.5	0.13 - 0.94
Bilirubin, indirect † (mg. per 100 cc.)	92	0.35 ± 0.01	0.16 ± 0.01	45.7	0.04 - 0.96
Leukocytes (number per cu. mm.)	167	6,800 ± 77	1,480 ± 54	21.8	3,480 - 14,840
Differential leukocyte count	70				
Neutrophils, stab (per cent)	...	2.6 ± 0.10	1.3 ± 0.07	50.0	0 - 6
Neutrophils, segmented (per cent)	...	55.1 ± 0.58	7.3 ± 0.41	13.2	38 - 70
Neutrophils, total (per cent)	...	57.8 ± 0.63	7.9 ± 0.45	13.7	38 - 72
Eosinophils (per cent)	...	4.2 ± 0.23	2.9 ± 0.17	69.0	0 - 15
Basophils (per cent)	...	0.6 ± 0.04	0.6 ± 0.03	100.0	0 - 2
Monocytes (per cent)	...	7.2 ± 0.20	2.5 ± 0.14	34.7	2 - 14.5
Lymphocytes (per cent)	...	29.8 ± 0.47	5.9 ± 0.34	19.8	18 - 49

* Colorimetric determination using a cobalt solution as standard.[5]
† Photoelectric colorimetric determination; technic of Malloy-Evelyn.[6]

TABLE 3.—*Observations on Blood of Healthy Adult Men Born at High Altitudes and Studied at Sea Level After a Residence of Six Months to a Year*

Determinations	Number of Subjects	Mean ± P. E.	St. Dev. ± P. E.	Coefficient of Variation (%)	Extreme Variations
Red blood cells (millions per cu. mm.)	25	5.00 ± 0.04	0.32 ± 0.03	6.4	4.46 - 5.85
Hematocrit (red cells, per cent)	25	44.0 ± 0.32	2.4 ± 0.23	5.4	37.5 - 49.1
Hemoglobin (grams per 100 cc.)	25	14.70 ± 0.13	1.00 ± 0.09	6.8	12.26 - 17.00
Corpuscular mean volume (cu. microns)	25	88.8 ± 0.62	4.6 ± 0.44	5.2	75.9 - 96.3
Corpuscular mean diameter (microns)	20	7.46 ± 0.03	0.20 ± 0.02	2.7	7.11 - 7.81
Corpuscular mean thickness (microns)	20	2.02 ± 0.02	0.11 ± 0.01	5.4	1.83 - 2.28
Corpuscular mean surface area (sq. microns)	20	133.6 ± 0.75	5.0 ± 0.53	3.7	124.7 - 143.5
Spherocytic index	20	0.28 ± 0.003	0.02 ± 0.002	7.1	0.24 - 0.32
Corpuscular mean hemoglobin (micromicrograms)	25	29.6 ± 0.29	2.2 ± 0.21	7.4	25.7 - 33.5
Corpuscular mean hemoglobin concentration (per cent)	25	33.4 ± 0.16	1.2 ± 0.11	3.6	31.7 - 36.3

21. Price-Jones, C.: Red Blood Cells Diameter, London, Oxford University Press, 1933.

22. Price-Jones, C.; Vaughan, J. M., and Goddard, H. M.: J. Path. & Bact. **40**:503, 1935.

23. Dameshek, W., and Singer, K.: Familial Nonhemolytic Jaundice: Constitutional Hepatic Dysfunction with Indirect van den Bergh Reaction, Arch. Int. Med. **67**:259 (Feb.) 1941.

24. Emmons, W. F.: J. Physiol. **64**:215, 1927.

25. Gram, H. C.: Acta med. Skandinav. **66**:295, 1927.

26. Guest, G. M., and Wing, M.: J. Clin. Investigation **21**:257, 1942.

27. Bernstein, M., and Chesluk, H. M.: J. Lab. & Clin. Med. **27**:1280, 1942.

surface area, figures which approximate our mean value, of 136.7 ± 0.22. It is interesting that this measurement, which is indirect and derived from others, has in our series of normal men a low coefficient of variation (2.8 per cent). Dameshek and Singer [23] have given the values of 0.25 to 0.33 as the normal range for the spherocytic index; our mean value of 0.28 ± 0.01 falls within this range.

We have observed in the series of healthy men studied at sea level that the variations in some of the morphologic characteristics of the circulating erythrocytes are related to the number of these cells per cubic millimeter (table 4). Although these variations are small and within the normal range, the observed relationship is of interest in connection with the findings at high altitudes, which will be discussed in another section.

There is some variation in the results obtained by several investigators in regard to the normal number of reticulated red cells in the peripheral blood, but with the exception of Osgood, Baker and Wilhelm,[28] who found a mean value of 1.5 per cent, all observed averages have been under 1 per cent, this being considered by most workers as the upper normal limit of variation. Our mean values of 0.5 ± 0.02 per cent and 18.4 ± 0.94 thousand per cubic millimeter, obtained for 93 men, are in agreement with such previous findings.

TABLE 4.—*Relationship Between the Number and the Morphologic Characteristics of the Red Blood Cells (Observations on 175 Healthy Men at Sea Level)*

Red Blood Cells, Millions per Cu. Mm.	Number of Subjects	Corpuscular Mean Volume ± P. E., Cu. Microns	Corpuscular Mean Hb ± P. E., Micromicrograms	Corpuscular Mean Hb Concentration ± P. E., per Cent
4.00 - 4.49	7	93.2 ± 0.88	34.1 ± 0.21	34.7 ± 0.16
4.50 - 4.99	48	94.8 ± 0.17	32.8 ± 0.15	34.6 ± 0.10
5.00 - 5.49	92	90.2 ± 0.22	30.7 ± 0.10	34.0 ± 0.10
5.50 - 5.99	28	87.1 ± 0.46	29.4 ± 0.15	33.6 ± 0.17

The serum bilirubin has been determined for 93 subjects in its total amount, by means of a colorimetric procedure and with a cobalt solution used as a standard for comparison,[5] and for another group, of 92 men, in its fractionated forms, direct and indirect, by the recently introduced photoelectric colorimetric method of Malloy and Evelyn.[6] There is a close agreement between the mean values of 0.76 ± 0.03 and 0.72 ± 0.02 mg. per hundred cubic centimeters obtained by these methods, respectively, for the total amount of bilirubin in the serum. It is difficult to evaluate these figures from the point of view of previous investigations, on account of the lack of uniformity among the reported values for the normal concentration of bilirubin, which has been estimated to vary between 0.10 and 3.50 mg. per hundred cubic centimeters [29]; most investigators have, however, shown a tendency to consider 1.00 mg. as the upper normal limit of variation. In our series of determinations this limit was frequently exceeded; of a total of 185 apparently healthy men investigated, 24, or 13 per cent, had a serum bilrubin above 1.00 mg. According to studies made by one of us (E.D.),[30] it is likely that these high bilirubin values observed at times in the blood of apparently normal subjects are due in most cases to an insufficiency of the liver in its function of excreting pigment. If this is confirmed, the range of bilirubin concentration in the plasma considered normal will have to be considerably reduced. Cantarow and others [31] have found that the direct bilirubin constitutes about 35 to 70 per cent of the total amount, with absolute values between 0.15 and 0.35 mg. Our mean value of 0.37 ± 0.01 and the standard deviation of 0.15 mg. are distinctly higher.

In regard to the fragility of the red blood cells to hypotonic solutions of sodium chloride, we obtained the mean values of 0.46 ± 0.001 and 0.38 ± 0.002 per cent for initial and total hemolysis, respectively, in determinations made for 38 of our subjects. Daland and Worthley,[32] in an investigation made on 20 normal adults, observed the first traces of hemolysis at concentrations of 0.44 to 0.47 per cent and its completion at 0.27 per cent; the latter low figure is probably accounted for by the microscopic technic employed.

The great variations which are frequently observed in apparently healthy subjects in regard to the number of leukocytes in the circulating blood have been emphasized by Garrey and Brown.[33] We have not observed the extreme

28. Osgood, E. E.; Baker, R. L., and Wilhelm, M. M.: Am. J. Clin. Path. **4**:292, 1934.

29. von den Bergh, H.: Die Gallenforbstoffe in Blute, Leipzig, 1918. Haselhorst, G.: München. med. Wchnschr. **68**:174, 1921. Förster, J.: Klin. Wchnschr. **4**:1689, 1925. Perkins, F. S.: Blood Bilirubin: Estimation and Clinical Significance, Arch. Int. Med. **40**: 195 (Aug.) 1927. Vaughan, J. M., and Haslewood, G. A. D.: Lancet **1**:133, 1938. Abels, J. C.; Rekers, P. E.; Binkley, G. E.; Pack, J. T., and Rhoads, C. P.: Ann. Int. Med. **16**:221, 1942. López García, A., and Zelasco J. F.: An. d. Inst. d. invest. fís. apl. a la pat. humana **3**:89, 1941.

30. Delgado, E.: Estudios sobre bilirrubina, Lima, Facultad de Medicina, 1943.

31. Cantarow, A.; Wirts, C. W., Jr., and Hollander, G.: Quantitative Studies of Direct-Reacting Serum Bilirubin, Arch. Int. Med. **69**:986 (June) 1942.

32. Daland, G. A., and Worthley, K.: J. Lab. & Clin. Med. **20**:1122, 1935.

33. Garrey, W. F., and Brown, R. W.: Physiol. Rev. **15**:597, 1935.

fluctuations found by some investigators.[34] Our mean value, of 6,800 ± 77, leukocytes per cubic millimeter, and the standard deviation, of 1,480, are almost identical with those observed by Price-Jones, Vaughan and Goddard [22] in 100 men in England and by Osgood and others [35] in 198 men in the United States. The differential leukocyte count, determined for 70 of our subjects, gave values which agree with those indicated by Schilling [9] but which are slightly different, in the percentage of stab neutrophils, eosinophils, monocytes and lymphocytes, from the findings of Osgood and others.[35]

Table 5 summarizes the mean values obtained by several investigators [36] in the determination of the circulating blood volume of healthy men, by means of various dye methods. Our findings, consisting of values for 36 subjects, for 26 of whom the determination was made with brilliant

TABLE 5.—*Determinations of Blood Volume in Healthy Men (by Dye Methods) at Sea Level and in Different Parts of the World—Summary of Literature* [36]

Investigators	Place	Dye Used	Number of Subjects	Blood, Cc. per Kg., Average Values	Plasma, Cc. per Kg., Average Values
Rowntree and Brown	U. S. A.	Congo-v. red	49	88.6	51.0
Gibson and Evans	U. S. A.	Evans blue	49	77.7	44.7
Looney and Freeman	U. S. A.	Congo red	29	81.4	44.1
Kaltreider, Hurtado and Brooks	U. S. A.	Brilliant vital red	25	79.1	42.8
Sparks and Haden	U. S. A.	Congo red	10	64.9	34.4
Goldbloom and Libin	U. S. A.	Trypan red	10	78.4	40.5
Hallock	U. S. A.	Evans blue	10	89.5	46.4
Levin	Argentina	Vital red	56	78.0	42.0
Hurtado, Pons and Merino	Peru	Brilliant vital red	15	87.8	47.8
Davis	England	Evans blue	11	76.7	40.5
Menderhaussen	Germany	Congo red	16	71.7	39.0
Rusznyák	Germany	Trypan red	8	83.3	44.7
Uhlenbruck	Germany	Congo red	7	79.6	41.4
Seyderhelm and Lampe	Germany	Trypan red	6	84.7	44.1
Grand mean ± P. E.				80.1 ± 1.20	43.1 ± 0.72

TABLE 6.—*Determinations of Blood Volume (by Dye Methods) in Healthy Men at Sea Level*

	Mean ± P. E.	St. Dev. ± P. E.	Coefficient of Variations (%)	Extreme Variations
	With Brilliant Vital Red (26 Subjects)			
Blood volume (liters)	5.21 ± 0.10	0.79 ± 0.07	15.2	3.96 - 6.60
Blood volume (cc. per Kg.)	86.5 ± 1.10	8.3 ± 0.77	9.6	72.2 - 106.4
Plasma volume (liters)	2.82 ± 0.06	0.43 ± 0.04	15.2	2.19 - 3.77
Plasma volume (cc. per Kg.)	47.1 ± 0.59	4.5 ± 0.42	9.6	36.3 - 56.4
Red cell volume (liters)	2.34 ± 0.03	0.36 ± 0.03	15.4	1.75 - 3.11
Red cell volume (cc. per Kg.)	38.8 ± 0.59	4.5 ± 0.42	11.6	30.8 - 47.0
Total hemoglobin (grams)	788 ± 14.8	112 ± 10.4	14.2	604 - 1,059
Total hemoglobin (Gm. per Kg.)	13.2 ± 0.18	1.4 ± 0.12	10.6	10.1 - 15.3
	With Evans Blue (10 Subjects)			
Blood volume (liters)	5.02 ± 0.19	0.85 ± 0.13	16.9	4.12 - 6.53
Blood volume (cc. per Kg.)	85.4 ± 1.28	5.7 ± 0.90	6.7	76.7 - 96.6
Plasma volume (liters)	2.70 ± 0.08	0.37 ± 0.05	13.7	2.21 - 3.47
Plasma volume (cc. per Kg.)	46.2 ± 1.03	4.6 ± 0.73	9.9	38.6 - 55.1
Red cell volume (liters)	2.29 ± 0.10	0.43 ± 0.07	18.8	1.73 - 3.21
Red cell volume (cc. per Kg.)	39.0 ± 0.49	2.2 ± 0.35	5.6	35.7 - 43.8
Total hemoglobin (grams)	779 ± 28.6	127 ± 20.2	16.3	653 - 1,057
Total hemoglobin (Gm. per Kg.)	13.3 ± 0.18	0.8 ± 0.12	6.0	12.0 - 14.5

34. (*a*) Feinblatt, H. M.: Alimentary Leukocytosis in Eighty Normal Men, J. A. M. A. **80**:613 (March 3) 1923. (*b*) Sabin, F. R.; Cunningham, R. S.; Doan, C. A., and Kindwall, S. A.: Bull. Johns Hopkins Hosp. **37**:14, 1925. (*c*) Looney, J. M., and Freeman, H.: Volume of Blood in Normal Subjects and in Patients with Schizophrenia, Arch. Neurol. & Psychiat. **34**:956 (Nov.) 1935.

35. Osgood, E. E.; Brownlee, I. E.; Osgood, M. W.; Ellis, D. M., and Cohen, W.: Total, Differential and Absolute Leukocyte Counts and Sedimentation Rates Determined for Healthy Persons 19 Years of Age and Older, Arch. Int. Med. **64**:105 (July) 1939.

36. (*a*) Looney and Freeman.[34c] Menderhaussen, A.: Ztschr. f. klin. Med. **97**:468, 1923. (*b*) Seyderhelm, R., and Lange, W.: Ztschr. f. d. ges. exper. Med. **35**:177, 1923. (*c*) Rusznyák, S.: Deutsches Arch. f. klin. Med. **157**:186, 1927. (*d*) Rowntree, L. G., and Brown, G. E.: The Volume of the Blood and Plasma in Health and Disease, Philadelphia, W. B. Saunders Company, 1924. (*e*) Uhlenbruck, P., and Vogels: Ztschr. f. klin. Med. **118**:172, 1931. (*f*) Sparks, M. I., and Haden, R. L.: Am. J. M. Sc. **184**:753, 1932. (*g*) Kaltreider, N. L.; Hurtado, A., and Brooks, W. D. W.: J. Clin. Investigation **13**:999, 1934. (*h*) Goldbloom, A. A., and Libin, I.: Clinical Studies in Circulatory Adjustments: Clinical Evaluation of Studies of Circulating Blood Volume, Arch. Int. Med. **55**:484 (March) 1935. (*i*) Gibson, J G., and Evans, W. A.: J. Clin. Investigation **16**:317, 1937. (*j*) Levin, E.: El volumen de la sangre circulante, Buenos Aires, El Ateneo, 1938. (*k*) Hallock, P.: Proc. Soc. Exper. Biol. & Med. **44**:11, 1940. (*l*) Davis, L. J.: Edinburgh M. J. **49**:465, 1942.

vital red [37] and for 10 by the recently introduced method using Evans blue,[38] are given in table 6. The study of these tables reveals a rather close similarity among the various listed mean values and suggests that, at least in healthy subjects, the results obtained by the older and the newer dye methods are fundamentally alike.[38a] It must be indicated, however, that the studies of Smith, Arnold and Whipple [39] and of Stead and Ebert,[40] who have pointed out that the cell-plasma ratio of the venous blood does not necessarily represent that of the entire circulation, have raised an objection, common to all dye methods, to the use of the hematocrit as a basis for the calculation of the total blood volume.

in Lima (at sea level) after a residence of less than one year in this place (table 3) shows a moderate, but definite, decrease in the number of red cells and the amount of hemoglobin in the latter group; this is not accompanied by alterations in the morphologic characteristics of the erythrocytes.

INFLUENCE OF TEMPORARY ANOXEMIA

It has been repeatedly observed that a temporary anoxemia brought about by a lowered oxygen tension in the inspired air or by interference with the mechanisms responsible for the acquisition of this gas at lung level is followed in

Chart 1.—Altitudes and location of the different places where the hematologic studies were carried out (zone located on the western slope of the Andes in the central region of Peru).

Comparison of the results obtained in the study of the 175 normal men with those for the group of 25 men born at high altitudes and investigated most cases by an increase in the number of red cells and the amount of hemoglobin in the circulating blood. There are, however pronounced individual variations in the polycythemic response, which may be related to the degree and duration of the anoxic stimulus and to individual factors not yet well understood. We have taken four different groups of healthy men (total 67) to altitudes of 2,390 meters (7,920 feet), 3,140 meters (10,300 feet), 4,165 meters (13,660 feet) and 4,835 meters (15,860 feet). Most of the subjects were medical students, of ages between 20 and 32 years; 36 were natives of sea level localities and 31 had been born in places located at altitudes over 2,000 meters (7,660 feet) but

37. Keith, Rowntree and Geraghty.[18] Hooper, Smith, Belt and Whipple.[14]

38. Gibson and Evans.[15] Evelyn and Gibson.[16]

38a. A statistical study of the several series of observations found in the literature concerning the plasma volume in healthy men shows the same degree of variation in the results obtained with the older and the newer dye methods (brilliant vital red and Evans' blue, respectively).

39. Smith, H. P.; Arnold, H. R., and Whipple, G. H.: Am. J. Physiol. **56**:1921, 1921.

40. Ebert, R. V., and Stead, E. A.: J. Clin. Investigation **20**:317, 1941.

with a residence of one or more years at sea level previous to the investigation. Blood was obtained at sea level immediately before the ascent and again within the first two hours after arrival at the given altitude. From 12 men, 3 in each of the high altitude places, the sample of blood was taken after three hours of moderate physical activity (walking). In addition, a fifth group, of 10 healthy men, also medical students, were taken from sea level to Morococha, at an altitude of 4,540 meters (14,900 feet), where 6 of them were investigated immediately after arrival from the point of view of possible changes in the total circulating blood volume and the remaining 4 were studied daily during a period of six days' residence at that altitude. In order to know the degree of anoxemia at which the hematologic observations were made, arterial blood was taken from several subjects at each one of the altitudes reached and analyzed for its oxygen saturation; the results obtained are given in table 7. The mean saturation ranged, from the lowest to the highest altitude, between 91.0 and 75.3 per cent. The results obtained in the different investigations have been summarized in table 8. In most cases there occurred a moderate rise in the red blood cells and hemoglobin; the average increases in the latter substance at the four altitudes, from the lowest to the highest, were 0.29, 0.13, 0.57 and 0.47 Gm. per hundred cubic centimeters of blood, which represented increases of 1.8, 0.8, 3.6 and 2.9 per cent respectively, over the sea level value. There were marked individual variations (chart 2). In 11 of the subjects no change was found, this being more frequent at the lowest altitudes. In 15 men, 5 at the lower altitudes and 10 at the highest two, an actual decrease occurred, reaching a maximal value of 0.75 Gm. in 1 subject studied at 3,140 meters; it was interesting to observe that in this group of 15 subjects were included 8 of the 12 men who engaged in physical activity after arrival at high altitudes. In the other 41 subjects, or 61 per cent of the total, the hemoglobin concentration rose under the influence of the acute anoxemia; the greatest increase was 1.70 Gm. per hundred cubic centimeters, found in 2 men at the altitudes of 4,165 and 4,835 meters respectively. There was no relationship between the degree of increase in hemoglobin and the place of birth of the men studied; at the two highest places we observed average increases of 0.46 and 0.49 Gm. per hundred cubic centimeters of blood in men who had been born at sea level and at high altitudes, respectively.

TABLE 7.—*Degree of Anoxemia in Relation to Results of Hematologic Studies Made on Arrival at Places of High Altitude*

Place	Altitude		Average Barometric Pressure, Mm. Hg	Number of Subjects	Arterial Oxygen Saturation, per Cent *	
	Meters	Feet			Mean ± P. E.	Extreme Variations
Matucana	2,390	7,920	581.4	5	91.0 ± 0.57	88.2 - 93.6
San Mateo	3,140	10,300	530.5	5	89.6 ± 0.74	86.2 - 92.5
Casapalca	4,165	13,660	470.8	5	80.2 ± 0.77	77.0 - 83.2
Morococha	4,540	14,900	446.0	15	78.9 ± 0.68	70.7 - 83.8
La Cima	4,835	15,860	433.8	7	75.3 ± 0.93	70.4 - 79.8

* Determinations made within the first two hours after arrival.

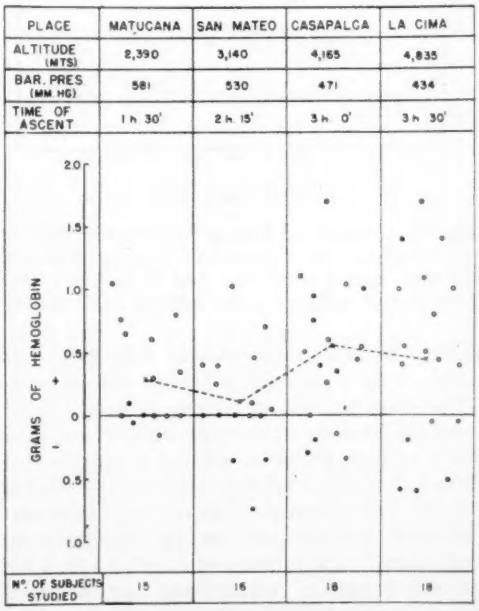

Chart 2.—Variations in the blood hemoglobin (expressed in grams per hundred cubic centimeters) in 67 healthy men studied within the first three hours after their arrival at high altitudes. Dots represent men who engaged in physical activity (walking) previous to the taking of the blood sample; circles, resting men.

Of the 36 men studied on arrival at the altitudes of 4,165 and 4,835 meters, 13 showed at the time when the blood was taken signs and symptoms of *soroche* (mountain sickness). The occurrence of this syndrome did not have a definite relationship to the level of hemoglobin before the exposure; in the men with *soroche* the hemoglobin at sea level had an average value of

15.68 Gm. per hundred cubic centimeters, as compared with 16.00 Gm. in the unaffected group. However, of 6 subjects who before exposure had a hemoglobin concentration below 15.00 Gm., 4 showed symptoms on arrival at high altitudes. The average increase in hemoglobin in the ill men was 0.40 Gm. per hundred cubic centimeters, and 5, or 38.5 per cent, showed a decrease at high altitudes. In the men without symptoms the

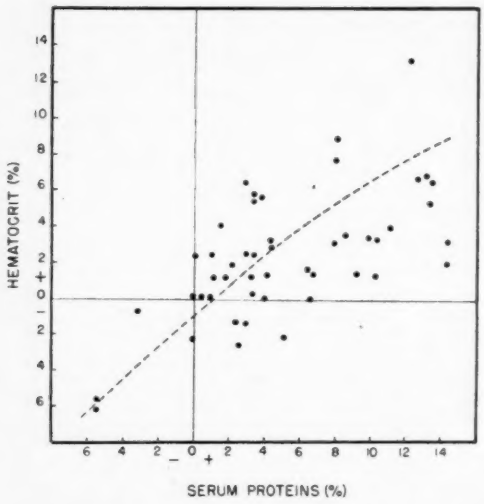

Chart 3.—Relationship between the percentage increase of serum proteins and of hematocrit value for 48 healthy adult men studied within the first three hours after arrival at high altitudes (from 2,390 to 4,835 meters).

average increase observed was 0.53 Gm., and only 4, or 17.4 per cent, exhibited a decrease.

The concentration of serum proteins, determined for 50 of the subjects at sea level and after arrival at high altitudes, showed a definite rise, which had a direct relationship with the level of altitude. The average observed increases were 0.19, 0.32, 0.48 and 0.64 Gm. per hundred cubic centimeters at the altitudes of 2,390, 3,140, 4,165 and 4,835 meters, respectively, which represented increases of 2.9, 4.0, 5.3 and 7.9 per cent over the sea level values. Some relationship was also observed between the degree of increase in the serum proteins and in the hematocrit value (cubic centimeters of red cells per hundred cubic centimeters of blood), but the rises in the latter (1.6, 1.5, 2.3 and 3.5 per cent, at the different altitudes, over the sea level values) were proportionately less than those observed in the protein substances (charts 3 and 4).

Although on arrival at high altitudes the men showed some individual changes in the morphologic characteristics of the red blood cells (mean corpuscular volume, hemoglobin and hemoglobin concentration), no definite trends were observed, and the mean values were almost identical with those obtained previously at sea level (table 8).

The number of reticulocytes determined in thousands per cubic millimeter of blood showed a very slight rise at the high altitudes, a finding related to the increase in the number of red cells; the percentage values did not vary. The serum bilirubin did not show a constant change during the short exposure to the low barometric pressure, with the exception of a slight mean rise in the direct fraction at the highest altitude (4,835 meters).

With a variation of 2,000 or more white cells per cubic millimeter from the sea level value—taken as an arbitrary index of a significant change in the leukocyte count on a subject's arrival at a high altitude—we found a rise of this nature to occur in 5, 7 and 8 men at the altitudes of 3,140, 4,165 and 4,835 meters respectively (which corresponded to 31.2, 38.9, and 44.4 per cent of the subjects studied at these altitudes). In 6 of these subjects the increased count exceeded 10,000 per cubic millimeter; the highest value, 16,040 leukocytes, occurred in a subject studied at 4,165 meters (chart 5). In 2 men a significant

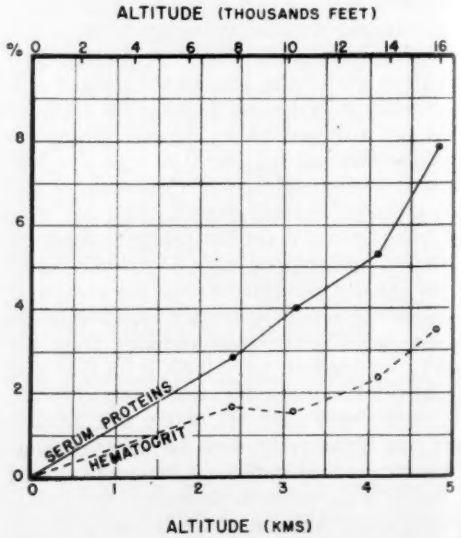

Chart 4.—Mean percentage increase in serum proteins and hematocrit value within the first three hours after arrival at altitudes of 2,390, 3,140, 4,165 and 4,835 meters. Observations made on healthy adult men.

decrease was observed, and no change took place at the lowest altitude. Physical activity and *soroche* were frequently associated with a rise in the leukocyte count; in 4 of the 6 men who exercised at the highest stations and in 8 of the 13 patients with *soroche* this change was observed. The frequent rise in the number of

TABLE 8.—*Observations on Blood Made in Lima (at Sea Level) and Immediately After Arrival at Several Places of High Altitude* (Four Different Groups of Men Studied)*

Place	Lima	Matucana	Lima	San Mateo	Lima	Casapalca	Lima	La Cima
Altitude (meters)	Sea level	2,390	Sea level	3,140	Sea level	4,165	Sea level	4,835
Number of subjects	15		16		18		18	
	Mean ± P. E.							
Red blood cells (millions per cu. mm.)	4.96 ± 0.06	4.97 ± 0.04	5.11 ± 0.06	5.14 ± 0.05	4.88 ± 0.05	5.05 ± 0.04	5.04 ± 0.04	5.17 ± 0.05
Hematocrit (red cells, per cent)	45.7 ± 0.43	46.5 ± 0.50	46.3 ± 0.31	46.8 ± 0.31	45.7 ± 0.36	46.9 ± 0.43	45.8 ± 0.24	47.7 ± 0.35
Hemoglobin (Gm. per 100 cc.)	15.93 ± 0.13	16.22 ± 0.16	16.18 ± 0.14	16.31 ± 0.15	15.63 ± 0.18	16.15 ± 0.15	16.06 ± 0.11	16.53 ± 0.18
Corpuscular mean volume (cu. microns)	92.0 ± 0.79	93.1 ± 0.50	90.8 ± 0.89	91.2 ± 0.57	93.7 ± 0.77	93.0 ± 1.28	91.3 ± 0.71	92.0 ± 0.51
Corpuscular mean Hb (micromicrograms)	32.2 ± 0.32	32.7 ± 0.29	31.7 ± 0.26	31.8 ± 0.17	32.2 ± 0.30	32.3 ± 0.46	32.0 ± 0.30	31.8 ± 0.27
Corpuscular mean Hb concentration (%)	34.8 ± 0.18	34.9 ± 0.16	35.0 ± 0.21	34.8 ± 0.19	34.5 ± 0.28	34.7 ± 0.19	35.0 ± 0.16	34.6 ± 0.16
Reticulocytes (per cent)	0.3 ± 0.03	0.5 ± 0.05	0.3 ± 0.05	0.5 ± 0.08	0.3 ± 0.03	0.4 ± 0.05	0.3 ± 0.03	0.4 ± 0.04
Reticulocytes (thousands per cu. mm.)	14.6 ± 2.19	24.6 ± 2.99	15.6 ± 2.84	24.9 ± 4.22	16.3 ± 1.27	19.8 ± 2.22	15.0 ± 2.18	21.8 ± 2.50
Serum proteins (Gm. per 100 cc.)	7.41 ± 0.06	7.60 ± 0.07	7.21 ± 0.05	7.53 ± 0.06	7.52 ± 0.06	8.00 ± 0.05	7.25 ± 0.06	7.89 ± 0.06
Bilirubin, direct (mg. per 100 cc.)	0.49 ± 0.04	0.45 ± 0.03	0.36 ± 0.02	0.36 ± 0.02	0.33 ± 0.01	0.36 ± 0.02	0.32 ± 0.02	0.39 ± 0.03
Bilirubin, indirect (mg. per 100 cc.)	0.31 ± 0.02	0.31 ± 0.02	0.28 ± 0.03	0.31 ± 0.03	0.38 ± 0.02	0.38 ± 0.03	0.38 ± 0.03	0.40 ± 0.04
Bilirubin, total (mg. per 100 cc.)	0.80 ± 0.04	0.76 ± 0.04	0.64 ± 0.05	0.67 ± 0.04	0.71 ± 0.03	0.74 ± 0.04	0.70 ± 0.06	0.79 ± 0.07
Leukocytes (thousands per cu. mm.)	6.98 ± 0.17	6.84 ± 0.17	7.05 ± 0.23	7.73 ± 0.26	6.94 ± 0.21	8.24 ± 0.41	6.28 ± 0.17	8.40 ± 0.29
Leukocytic differential count:								
Neutrophils, stab (%)	2.5 ± 0.20	1.8 ± 0.24	1.6 ± 0.24	2.1 ± 0.26	2.4 ± 0.19	1.8 ± 0.16	2.9 ± 0.29	2.8 ± 0.29
Neutrophils, segmented (%)	55.8 ± 1.35	57.3 ± 1.87	54.3 ± 1.55	61.9 ± 1.72	53.9 ± 1.21	57.1 ± 1.81	54.1 ± 0.99	62.0 ± 1.91
Neutrophils, total (%)	58.3 ± 1.51	59.1 ± 1.94	55.9 ± 1.55	64.0 ± 1.77	56.3 ± 1.19	58.7 ± 1.65	57.0 ± 1.03	64.8 ± 2.00
Eosinophils (%)	4.9 ± 0.73	4.8 ± 0.86	3.5 ± 0.60	2.4 ± 0.31	4.2 ± 0.49	4.6 ± 0.33	3.6 ± 0.47	3.3 ± 0.49
Basophils (%)	0.6 ± 0.09	0.4 ± 0.09	0.6 ± 0.09	0.8 ± 0.11	0.5 ± 0.09	0.5 ± 0.09	0.8 ± 0.10	0.6 ± 0.15
Monocytes (%)	6.9 ± 0.48	5.4 ± 0.26	6.9 ± 0.44	4.8 ± 0.35	7.1 ± 0.41	6.8 ± 0.47	7.2 ± 0.50	3.1 ± 0.47
Lymphocytes (%)	29.3 ± 0.95	30.3 ± 1.61	33.1 ± 1.21	28.0 ± 1.42	32.2 ± 0.99	30.0 ± 1.43	31.3 ± 0.56	28.3 ± 1.44

* The time of ascent from sea level to the different altitudes was as follows: Lima to Matucana, 1 hour, 30 minutes; Lima to San Mateo, 2 hours, 15 minutes; Lima to Casapalca, 3 hours; Lima to La Cima, 3 hours, 30 minutes.

leukocytes on the subjects' arrival at high altitudes was almost entirely due to an increase in the segmented neutrophils; the monocytes and the lymphocytes showed a proportional decrease. No constant changes were observed in the number of stab neutrophils, eosinophils and basophils. The occurrence of a temporary leukocytosis under the influence of a low pressure environment has been already reported. Meyer, Seevers and Beatty,[41] working with rats, and Sokolov [42] experimenting with cold-blooded animals, have observed such a hematic response in experiments carried out in chambers.

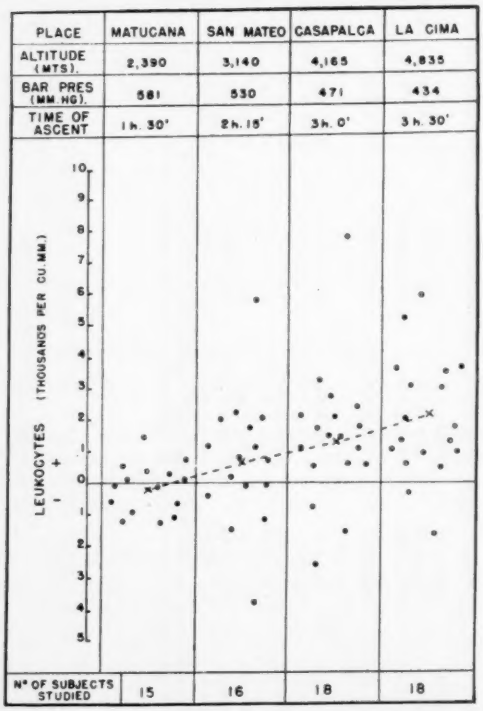

Chart 5.—Variation in the leukocyte counts (expressed in thousands per cubic millimeter) of 67 healthy men studied within three hours after arrival at high altitudes. Dots represent men who engaged in physical activity (walking) previous to the taking of the blood sample; circles, resting men.

In order to study further the mechanisms which are related to the polycythemic response to a condition of acute anoxemia we made determinations of blood volume for 6 subjects (medical students) within the first two hours after their arrival at Morococha, at an altitude of 4,540 meters (14,900 feet). Previous determinations had been made at sea level a few days before the ascent. The results obtained are given in table 9 and presented, graphically, in chart 6. The plasma volume remained unchanged in 2 subjects and decreased in the other 4; the average reduction in volume was 0.26 liters, equivalent to 10.5 per cent. The red cell volume increased in 5 subjects, and the elevation averaged 0.32 liters, or 13.8 per cent. In 3 of the men the total serum proteins rose on arrival at high altitudes; the increase had an average value of 10 Gm., equal to 8.2 per cent. In 1 subject a decrease of 13 Gm. (7.9 per cent) was found, and in the other 2 no significant change was verified.

The subsequent changes which take place in the circulating blood when the anoxic stimulus is prolonged for a few days were studied in a group of 4 men (medical students of ages varying between 21 and 25 years) who were taken from sea level to Morococha (at an altitude of 4,540 meters [14,900 feet]), where they remained for six days. The various investigations made previously at sea level were repeated on their arrival and then daily during the time of their residence at high altitudes. Determinations of blood volume were also made. The findings are given in tables 10 and 11. The moderate increase in the red blood cells and hemoglobin observed on the men's arrival was followed by a gradual rise, while the serum proteins, found to be sharply elevated in the first observation made at high altitudes, remained about unchanged, with a slight tendency to decrease (chart 7). One day after the men arrived the reticulocytes began to show an increase, which in the sixth, and last, day of study reached values of 2.2 to 4.0 per cent. This finding indicated a definite response of the bone marrow to the anoxic stimulus. In 3 subjects there was a moderate decrease in the mean corpuscular volume, while the mean corpuscular hemoglobin concentration remained practically unchanged. The plasma bilirubin, directly and indirectly determined, and total amount, did not show a significant variation except in 1 subject, who at sea level, before the ascent, had an abnormally high concentration of pigment. This subject three days after arrival at high altitudes had a considerable rise in the directly and indirectly determined bilirubin, with a subsequent decrease but without a decline to the initial value. It was interesting to observe in all subjects twenty-four hours after arrival a temporary and moderate rise in the leukocytes (chart 8) which was simultaneous with the beginning of the hyperactivity of the bone marrow as shown by the rise of the reticulocytes. The increase was almost entirely due to an elevated number of segmented neutrophils; there was no change in the stab neutrophils. The determinations of blood volume made after two, four and six days of residence

41. Meyer, O. O.; Seevers, M. H., and Beatty, S. R.: Am. J. Physiol. **113**:166, 1935.

42. Sokolov, A. N.: J. Med., Kiev **11**:145, 1941.

at high altitudes (chart 6) showed, in comparison with the values obtained at sea level, an unchanged plasma volume in 3 subjects, an increased volume in 1 (13.7 per cent) and a decreased volume in another (10.9 per cent). The cell volume was elevated in all subjects (12.0 to 43.4 per cent), and the total serum proteins showed increases in all of them (4.3 to 28.0 per cent). Increases, which fluctuated between 133 and 243 Gm., were found in the total amount of circulating hemoglobin. As a whole, all these changes had some proportional relationship to the length of residence at high altitudes.

Our observations concerning the changes which take place in the circulating blood volume during a short period of residence at high altitudes are not strictly comparable with those made by Smith, Belt, Arnold and Carrier [43] at an altitude of 3,350 meters. These investigators found no abrupt changes within the first day or two at that altitude; after two or three weeks an average increase of 19 per cent in the red cell volume was verified; the plasma volume did not show any significant variation. In 1913 Douglas,

TABLE 9.—*Determinations of Blood Volume Made at Sea Level and Within the First Two Hours After Arrival at Morococha (at 4,540 meters; 14,900 feet)* *

Subjects	Blood Volume (Liters)			Plasma Volume (Liters)			Red Cell Volume (Liters)			Total Hemoglobin (Grams)			Total Serum Protein (Grams)		
	Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %
1	4.28	3.92	—8.4	2.35	1.95	—17.0	1.91	1.95	+ 2.1	685	668	— 2.5	165	152	— 7.9
2	4.24	4.53	+6.8	2.36	2.15	— 8.9	1.86	2.35	+20.8	670	743	+10.9	169	171	+ 1.2
3	4.49	4.76	+6.0	2.49	2.52	+ 1.2	1.98	2.22	+10.8	678	752	+10.9	178	193	+ 8.4
4	4.71	5.16	+9.6	2.30	2.35	+ 2.2	2.39	2.79	+16.7	782	893	+14.2	162	180	+11.1
5	5.19	5.24	+1.0	2.85	2.65	— 7.0	2.31	2.56	+12.8	781	859	+10.0	185	186	+ 0.4
6	4.81	4.78	—0.6	2.70	2.46	— 8.9	2.09	2.30	+10.0	721	784	+ 8.7	181	190	+ 5.0

* Average time of ascent, 3 hours.

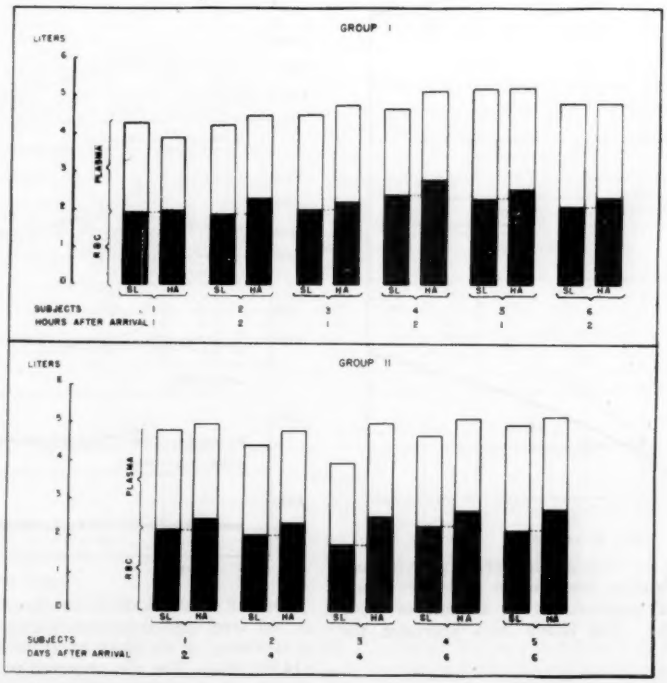

Chart 6.—Results of determinations of blood volume made for 11 healthy adult men at sea level and repeated at high altitudes (at 4,540 meters; 14,900 feet). Group I, determinations made one to two hours after their arrival; Group II, determinations made a few days after their arrival. The black zone represents red cell volume; the white zone, plasma volume.

43. Smith, H. P.; Belt, A. E.; Arnold, H. R., and Carrier, E. B.: Am. J. Physiol. **71**:395, 1925.

Haldane, Henderson and Schneider,[44] working at Pike's Peak, Colorado, at an altitude of 4,300 meters, and employing the carbon monoxide method, observed a significant rise in the blood

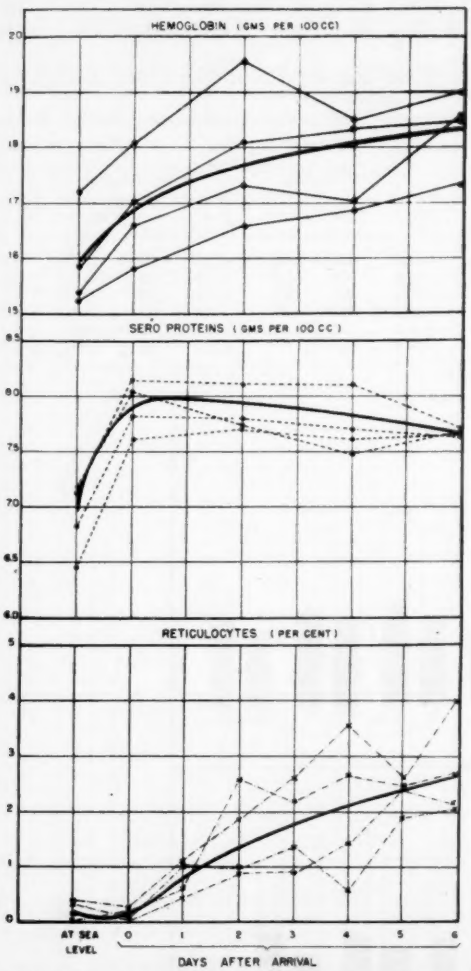

Chart 7.—Values for hemoglobin, serum proteins and reticulocytes in 4 healthy men studied at sea level and during six days of residence at an altitude of 4,540 meters (14,900 feet). The heavy lines represent the average values.

volume of 3 of the 4 subjects after a residence of nine to ten days. Laquer [45] found an increase of 5 per cent in the blood volume after four weeks of residence at an elevation of 1,560 meters.

INFLUENCE OF INTERMITTENT ANOXEMIA

The study of the effects produced on the body by intermittent periods of anoxemia, such as those experienced by pilots and other flight personnel, has attained a considerable interest in the last few years. Attention has been given to the psychologic aspects as well as to the adaptative responses of the respiratory and circulatory systems. Few investigations have been made concerning the morphologic response of the blood.

Meyer and Seyderhelm,[46] in 1916, studied the blood of 28 men who had been engaged in flying for a year or more and found in all cases an increase in the red blood cells and hemoglobin. McFarland, Graybiel, Liljencrantz and Tuttle,[47] in an analysis of the data collected in the examination of 200 civil air line pilots, the vast majority of whom had a record of flying at altitudes not over 3,050 meters (10,000 feet), found a red cell count above 6,000,000 in 50 per cent of them; the hemoglobin did not show a parallel

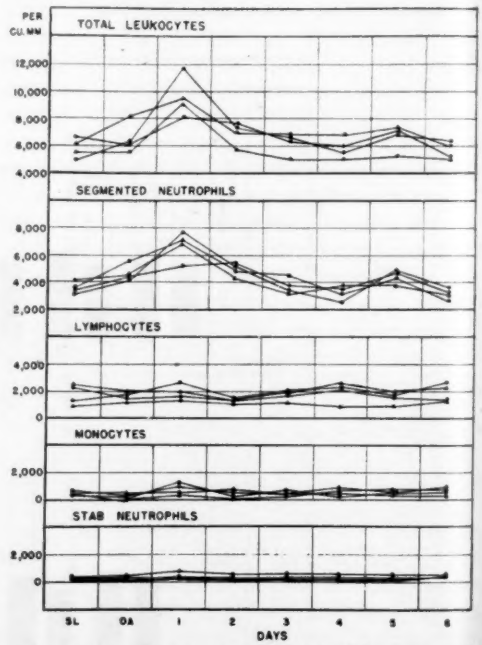

Chart 8.—Leukocyte counts for 4 healthy men studied at sea level and immediately after arrival and during a residence of six days at an altitude of 4,540 meters (14,900 feet). The rise observed twenty-four hours after their arrival corresponded to the beginning of the reticulocytosis (chart 7).

increase. Armstrong [48] and Heim observed that exposure for a period of four hours for three consecutive days to a simulated altitude of 3,660

44. Douglas, C. G.; Haldane, J. S.; Henderson, Y., and Schneider, E. C.: Philos. Tr. Roy. Soc., London, s.B **203**:185, 1913.

45. Laquer, F.: Klin. Wchnschr. **3**:7, 1924.

46. Meyer, E., and Seyderhelm, R.: Deutsche med. Wchnschr. **42**:1245, 1916.

47. McFarland, R. A.; Graybiel, A.; Liljencrantz, E., and Tuttle, A. D.: J. Aviation Med. **10**:160, 1939.

48. Armstrong, H. G.: Principles and Practice of Aviation Medicine, Baltimore, Williams & Wilkins Company, 1939.

TABLE 10.—*Observations on Blood of Four Men During a Six Day Residence in Morococha (4,540 Meters; 14,900 Feet)*

Observations	Subject 1								Subject 2							
			Days at High Altitudes								Days at High Altitudes					
	At S.L.*	O.A.†	1	2	3	4	5	6	At S.L.*	O.A.†	1	2	3	4	5	6
Red blood cells (mill. per cu. mm.).	4.99	4.99	5.53	5.71	5.57	5.63	5.28	5.45	4.78	4.62	5.11	5.48	5.03	4.88	4.95	4.96
Hemoglobin (Gm. per 100 cc.).....	15.4	16.6	....	17.3	....	17.1	....	18.6	15.3	15.8	....	16.6	....	16.8	....	17.3
Hematocrit (red cells, per cent)....	46.4	46.8	....	49.6	....	49.0	....	54.5	44.2	45.7	....	48.8	....	50.0	....	49.1
Reticulocytes (per cent).....	0.4	0	0.4	1.0	0.8	1.4	2.4	2.2	0.4	0	1.2	0.8	1.4	0.6	1.8	2 2
Reticulocytes (thous. per cu. mm.)	20.0	0	22.1	57.1	44.6	18.8	126.7	120.0	19.1	0	61.3	43.8	70.4	29.3	89.1	109.1
Bilirubin, total (mg. per 100 cc.)...	0.66	0.57	....	0.68	....	0.77	....	0.72	0.90	0.72	....	0.63	....	0.70	....	0.57
Bilirubin, direct (mg. per 100 cc.)...	0.40	0.37	....	0.45	....	0.47	....	0.33	0.50	0.45	....	0.49	....	0.52	....	0.35
Bilirubin, indirect (mg. per 100 cc.)	0.26	0.20	....	0.23	....	0.30	....	0.39	0.40	0.27	....	0.14	....	0.18	....	0.22
Leukocytes (thous. per cu. mm.)...	6.68	6.08	8.08	7.72	6.40	6.00	7.16	5.28	5.00	6.16	11.8	7.36	6.44	5.60	7 08	6 44
Serum protein (Gm. per 100 cc.)....	7.14	8.08	....	7.76	....	7.47	....	7.72	6.83	7.88	....	7.74	....	7.72	....	7.68

Observations	Subject 3								Subject 4							
Red blood cells (mill. per cu. mm.).	5.25	5.74	5.84	5.80	6.11	6.32	5.80	6.49	4.91	5.35	5.78	5.58	6.25	5.72	5.80	6.25
Hemoglobin (Gm. per 100 cc.).....	17.2	18.1	....	19.6	....	18.5	....	19.0	15.9	17.1	....	18.1	....	18.4	....	18.5
Hematocrit (red cells, per cent)....	49.2	50.7	....	53.4	....	53.9	....	52.8	43.9	45.7	....	50.7	....	49.2	....	52.0
Reticulocytes (per cent).....	0	0.2	1.2	1.8	2.6	3.6	2.6	4.0	0.2	0.2	0.6	2.6	2.2	2.6	2.4	2.6
Reticulocytes (thous. per cu. mm.)	0	11.5	70.1	104.4	158.9	227.5	150.8	259.6	9.8	10.7	34.7	145.1	137.5	148.7	141.4	162.5
Bilirubin, total (mg. per 100 cc.)...	1.03	0.68	....	0.97	....	0.88	....	0.72	1.34	1.52	....	2.56	....	1.90	....	1.90
Bilirubin, direct (mg. per 100 cc.)...	0.45	0.30	....	0.37	....	0.54	....	0.33	0.47	0.68	....	1.14	....	0.85	....	0.85
Bilirubin, indirect (mg. per 100 cc.)	0.58	0.38	....	0.60	....	0.34	....	0.39	0.87	0.84	....	1.42	....	1.05	....	1.05
Leukocytes (thous. per cu. mm.)...	5.60	5.64	9.04	5.88	5.12	5.08	5.16	5.04	6.24	8.16	9.60	7.68	6.96	6.96	7.08	6.16
Serum protein (Gm. per 100 cc.)....	7.19	8.14	....	8.12	....	8.12	....	7.66	6.45	7.62	....	7.72	....	7.61	....	7.68

* At S. L. = at sea level.
† O. A. = on arrival at high altitude.

TABLE 11.—*Determinations of Blood Volume at Sea Level and After a Few Days of Residence in Morococha (at 4,540 meters; 14,900 feet)*

Subjects	Days in Morococha	Blood Volume (Liters)			Plasma Volume (Liters)			Red Cell Volume (Liters)			Total Hemoglobin (Grams)			Total Serum Proteins (Grams)		
		Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %	Sea Level	Morococha	Variation, %
1	2	4.80	4.97	+ 3.5	2.60	2.51	— 3.5	2.17	2.43	+12.0	737	870	+18.0	185	193	+ 4.3
2	4	4.42	4.81	+ 8.8	2.35	2.43	+ 3.3	2.05	2.36	+15.1	678	820	+20.9	168	182	+ 8.3
3	4	3.96	5.03	+27.0	2.19	2.49	+13.7	1.75	2.51	+43.4	604	847	+40.2	150	192	+28.0
4	6	4.67	5.16	+10.5	2.35	2.41	+ 2.5	2.30	2.72	+18.2	803	980	+22.0	169	185	+ 9.5
5	6	4.96	5.18	+ 4.4	2.76	2.46	—10.9	2.18	2.69	+23.4	786	958	+21.9	178	189	+ 6.2

TABLE 12.—*Observations on Blood of Flight Personnel*

	Number of Subjects	Mean ± P. E.	St. Dev. ± P. E.	Coefficient of Variation, %	Extreme Variations
Red blood cells (millions per cu. mm.)	60	5.02 ± 0.04	0.51 ± 0.03	10.1	3.78 - 6.25
Hematocrit (red cells, per cent)	60	47.4 ± 0.25	2.9 ± 0.18	6.1	38.1 - 53.0
Hemoglobin (Gm. per 100 cc.)	60	16.53 ± 0.09	1.11 ± 0.07	6.7	14.10 - 19.10
Corpuscular mean volume (cu. microns)	60	94.3 ± 0.56	6.4 ± 0.39	6.8	77.5 - 107.4
Corpuscular mean hemoglobin (micromicrograms)	60	33.0 ± 0.18	2.1 ± 0.13	6.4	27.2 - 37.3
Corpuscular mean hemoglobin concentration (%)	60	35.1 ± 0.18	2.1 ± 0.13	6.0	31.9 - 37.9
Reticulocytes (per cent)	60	0.7 ± 0.05	0.6 ± 0.03	85.7	0 - 3.0
Reticulocytes (thousands per cu. mm.)	60	35.7 ± 2.63	30.2 ± 1.86	84.7	0 - 161.4
Bilirubin, total (mg. per 100 cc.)	45	1.00 ± 0.06	0.63 ± 0.04	63.0	0.35 - 3.49
Bilirubin, direct (mg. per 100 cc.)	45	0.43 ± 0.02	0.18 ± 0.01	41.9	0.12 - 1.05
Bilirubin, indirect (mg. per 100 cc.)	45	0.56 ± 0.05	0.55 ± 0.04	98.2	0.10 - 2.67
Leukocytes (per cu. mm.)	60	6,450 ± 137	1,580 ± 97	24.5	3,400 - 11,100
Leukocytic differential count	45	.....	.....	....	.....
Neutrophils, stab (per cent)	..	4.0 ± 0.21	2.1 ± 0.15	52.5	0 - 9
Neutrophils, segmented (per cent)	..	56.8 ± 0.79	7.9 ± 0.56	13.9	36 - 73
Neutrophils, total (per cent)	..	60.6 ± 0.82	8.2 ± 0.58	13.5	41 - 80
Eosinophils (per cent)	..	3.6 ± 0.23	2.3 ± 0.16	63.9	0 - 9.5
Basophils (per cent)	..	0.4 ± 0.06	0.6 ± 0.04	150.0	0 - 3
Monocytes (per cent)	..	6.0 ± 0.33	3.3 ± 0.23	55.0	0 - 14.5
Lymphocytes (per cent)	..	29.6 ± 0.78	7.8 ± 0.55	26.3	14 - 50

meters (12,000 feet) resulted in a decrease of the hemoglobin and the red cell count. Frequent exposures to carbon monoxide, which causes anoxemia by displacement of the oxygen from the circulating hemoglobin, at the same time decreasing the affinity of this substance for the latter gas,[49] has been found to produce a polycythemic response.[50] In a recent investigation, Stickney and Van Liere [51] observed a rise in the red blood cells and hemoglobin in dogs exposed six to nine hours daily for six months to simulated altitudes between 3,660 meters (12,000

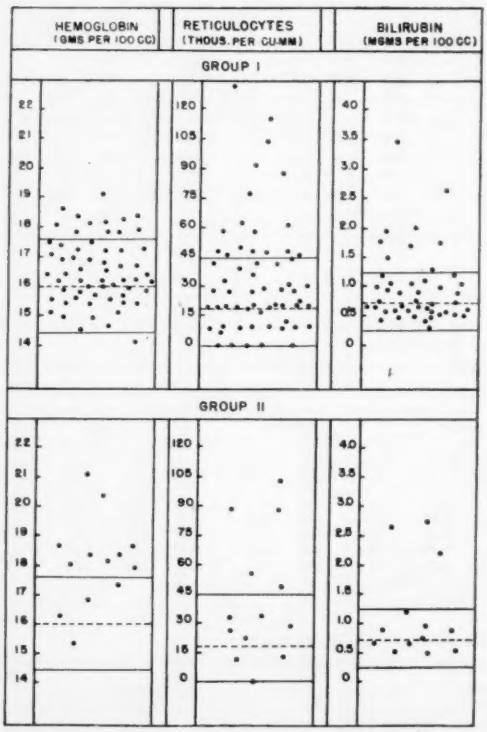

Chart 9.—Values for hemoglobin, reticulocytes and bilirubin in men exposed to intermittent periods of anoxemia. Group I, flight personnel; group II, railroad personnel (see text). The zones between the horizontal lines show the limits of variation observed in healthy men at sea level.

feet) and 4,880 meters (16,000 feet); these investigators concluded that discontinuous exposure to anoxia is capable of producing a noticeable acclimatization, the degree of which, in terms of increase in hemoglobin and erythrocytes, is proportional to the severity of the anoxia and the length of time of exposure.

49. Haldane, J. S., and Priestley, J. G.: Respiration, London, Oxford University Press, 1935.

50. Killick, E. M.: Physiol. Rev. **20**:313, 1940.

51. Stickney, J. C., and Van Liere, E. J.: J. Aviation Med. **13**:170, 1942.

Our studies include observations made on two groups of healthy men, both subject to the influence of intermittent periods of anoxemia but different from each other in regard to the length of time of exposure.

Observations on Flight Personnel.—Hematologic studies were carried out for 60 men, members of the flight personnel of a commercial air line which operates in South America; 47 of these men were pilots or co-pilots, 10 radio operators and 3 pursers. Fifty-seven had been born in the United States and 3 in Peru. Their ages varied between 21 and 49 years, with an average of 29 years. The total flying time of each man fluctuated between two hundred and several thousand hours, with a monthly average of sixty to ninety hours. Some of the routes covered by this air line require flying for several hours at altitudes over 3,660 meters (12,000 feet); most of the men reported the use of oxygen while flying over that level. The examinations were made from one to fourteen days after the previous trip. The results obtained are given in table 12.

The mean values of the hemoglobin content (grams per hundred cubic centimeters) and the hematocrit reading (percentage of red cells) were slightly higher than those obtained for the group of healthy men not subject to the influence of a low barometric pressure. Study of the individual findings (chart 9) revealed that 14 (23.3 per cent) of the 60 men had a hemoglobin concentration of 17.5 Gm. or more; the highest observed value was 19.10 Gm. Twelve men (20 per cent) had hematocrit values of 50 per cent or more, the highest being 53. Two subjects (3.3 per cent of the total) were found to have hemoglobin values of 14.1 and 14.5 Gm. per hundred cubic centimeters, with corresponding hematocrit readings of 38.1 and 40.4 per cent red cells; these values were the lowest found in this series of investigations. There was no correlation between the blood findings and the time spent at sea level after the previous trip; the latter had a similar average value of six days for the groups of men with hemoglobin concentrations above and below 17 Gm.

The mean corpuscular volume, of 94.3 ± 0.56 cubic microns, of the flight personnel was slightly higher than the corresponding value (91.3 ± 0.23) for the control group studied at sea level; in 12 cases (20 per cent) the volume exceeded 100 cubic microns. The mean corpuscular hemoglobin concentration ranged within normal limits. The reticulocytes, expressed in percentage and in number per cubic millimeter, had higher mean values in the group of fliers; 15 (25 per cent) of the 60 men studied had one or more red blood cells per hundred that appeared to be reticulated.

The serum bilirubin, determined for 45 men of the flight personnel, showed significant variations. The mean values, of 0.43 ± 0.02, 0.56 ± 0.05 and 1.00 ± 0.06 mg. per hundred cubic centimeters, for the directly and indirectly determined and total bilirubin, respectively, were higher than the corresponding values found in the blood of the subjects we studied for comparative purposes. The increase was more marked in the indirect (51.3 per cent) than in the direct value (22.8 per cent); this lowered the ratio direct/total bilirubin, which in the flight personnel had an average value of 43 per cent, as compared with 51.4 per cent in the nonfliers. Seventeen of the 45 men studied, or 37.8 per cent, had a total bilirubin content of over 1 mg., while this was found in only 13 per cent of the spent alternate nights at sea level and at an altitude of 3,730 meters (12,340 feet), traveling daily for several hours, one day on ascent and the next on descent, without taking oxygen, along a road whose highest point is at an altitude of 4,740 meters (15,510 feet). Observations were made on 13 of these men during one of the brief periods they were in Lima, at sea level; their ages varied between 23 and 54 years. Ten had been born at high altitudes and 3 at sea level. The time during which they had been intermittently exposed to a low barometric pressure, with the exception of one month's vacation each year, fluctuated between one and twenty-eight years. The results found in the different investigations, which included determinations of blood volume for 11 of the men, are given in tables 13 and 14.

TABLE 13.—*Observations on Blood of Men Daily Exposed to a Low Barometric Pressure (Railroad Personnel)*

Subject	Age, Years	Years of Exposure	Red Blood Cells, Millions per Cu. Mm.	Hematocrit, Red Blood Cells, per Cent	Hemoglobin, Gm. per 100 Cc.	Mean Corpuscular Volume, Cu. Microns	Mean Corpuscular Hemoglobin, Micromicrograms	Mean Corpuscular Hemoglobin Concentration, per Cent	Reticulocytes		Bilirubin, Mg. per 100 Cc.		
									Per Cent	Thousands per Cu. Mm.	Total	Direct	Indirect
1	44	28	5.61	51.4	18.60	91.6	33.1	36.1	1.6	89.8	0.86	0.45	0.41
2	54	25	6.25	56.6	20.35	90.6	32.6	36.0	1.4	87.5	0.88	0.33	0.55
3	42	20	6.49	60.6	21.10	93.4	32.5	34.8	0.4	26.0	2.74	0.86	1.88
4	48	20	5.15	51.6	18.00	100.2	34.9	34.8	1.0	51.5	0.68	0.33	0.35
5	49	13	5.40	48.6	17.30	90.0	32.0	35.5	0.4	21.6	0.52	0.23	0.29
6	41	12	4.81	47.0	16.25	97.7	33.8	34.6	0.6	28.9	0.50	0.26	0.24
7	36	9	5.33	50.7	18.60	95.1	34.8	36.6	0.6	32.0	1.20	0.36	0.84
8	36	7	4.68	50.6	16.75	108.1	35.8	33.1	1.2	56.2	0.75	0.33	0.42
9	40	4	5.14	53.2	18.10	103.5	35.2	34.0	2.0	102.8	0.50	0.28	0.22
10	38	3	5.53	52.3	17.90	94.6	32.4	34.2	0.2	11.1	2.19	0.50	1.69
11	23	3	5.74	52.8	18.35	92.0	32.0	34.8	0.2	11.5	0.66	0.32	0.34
12	39	1	4.68	44.1	15.30	94.2	32.7	34.7	0	0	0.93	0.47	0.46
13	29	1	5.63	54.1	18.30	96.1	32.5	33.8	0.6	33.8	2.66	0.43	2.23
Mean ± P. E.			5.42 ± 0.10	51.8 ± 0.76	18.07 ± 0.28	95.9 ± 0.99	33.4 ± 0.25	34.8 ± 0.18	0.8 ± 0.11	42.5 ± 6.16	1.16 ± 0.15	0.40 ± 0.06	0.76 ± 0.13
St. Dev. ± P. E.			0.53 ± 0.07	3.9 ± 0.54	1.47 ± 0.20	5.1 ± 0.62	1.3 ± 0.17	0.9 ± 0.12	0.6 ± 0.08	31.6 ± 4.36	0.78 ± 0.10	0.15 ± 0.02	0.67 ± 0.09
Coefficient of variation, %			9.8	7.5	8.1	5.3	3.9	2.6	75.0	74.4	67.2	37.5	88.1

group studied at sea level; the high bilirubin values of 11 of the 17 subjects corresponded to an elevation in the indirectly determined pigment, and in the other 6 the directly determined bilirubin was responsible for the rise, though in a lesser degree.

The numbers of leukocytes per cubic millimeter and the differential leukocyte counts of the flight personnel did not show any significant variation when compared with the results obtained in the study of the men living constantly at sea level.

Observations on Men Daily Exposed to a Low Barometric Pressure.—We had the opportunity to study a small group of men who occupied a perhaps unique position in regard to an intermittent exposure to a low barometric pressure. These men were members of the crew personnel of a railroad which connects Lima, at sea level, with high localities on the Andean plateau (chart 1), and for a prolonged time they had

A rise in the hemoglobin content and in the hematocrit readings was frequently observed; the mean values, of 18.07 ± 0.28 Gm. per hundred cubic centimeters and 51.8 ± 0.76 per cent red cells, respectively, were distinctly higher than those obtained for healthy males living constantly at sea level. The hemoglobin concentrations of 9 of the men (69.2 per cent) exceeded 17.50 Gm., and those of 2 of them reached the figures of 20.35 and 21.10 Gm. (chart 9). The hematocrit readings of 10 of the subjects (76.9 per cent) was over 50.0 per cent; the highest observed value was 60.6 per cent. There was not a constant direct relationship between the time of exposure to the high altitude and the degree of polycythemia; however, the three highest hemoglobin values, 18.60, 20.35 and 21.10 Gm. per hundred cubic centimeters, were found in the men who had the most prolonged time of service in the railroad, twenty-eight, twenty-five and twenty years, respectively.

The mean corpuscular volume, of 95.9 ± 0.99 cubic microns, was slightly higher than the one obtained for the group of men studied at sea level. The mean corpuscular hemoglobin concentration ranged within normal limits. The reticulocytes, in per cent and per cubic millimeter, also showed higher mean values than in men not exposed to high altitudes; in 5 cases (38.5 per cent) the percentage value fluctuated between 1.0 and 2.0. The plasma bilirubin was found to exceed 1.00 mg. per hundred cubic centimeters in 5 of the men (38.5 per cent); this rise was due exclusively to a higher amount of the indirectly determined pigment, which had a mean increase of 117.1 per cent over the corresponding normal value at sea level. The ratio direct/total bilirubin had an average value of 34.5 per cent.

The number of leukocytes per cubic millimeter had a normal mean value; in only 1 case it was moderately elevated (11,900). The differential leukocyte count, made for 12 of the subjects, revealed a frequent and moderate increase in the segmented neutrophils with a corresponding decrease in the monocytes and lymphocytes, a change almost identical to the one found in men exposed to a temporary period of anoxemia (table 8).

The determinations of blood volume, made for 11 of the men, showed well defined changes (chart 10). The red cell volume, expressed in cubic centimeters per kilogram of body weight, was elevated in 7 of the men (63.6 per cent); its mean value, of 46.5 ± 1.14 cc., represented an increase of almost 20 per cent over the corresponding normal value at sea level. The plasma volume, expressed similarly, was decreased in 4 and unchanged in 7 subjects; its mean value was 10.2 per cent lower than in men not exposed to a low barometric pressure. The circulating hemoglobin, in grams per kilogram of body weight, showed an average increase of 22.7 per cent and was found to be elevated in 9 of the 11 men studied (81.8 per cent).

Five men had a history of occasionally having mild headache and a sensation of dizziness while traveling at high altitudes. When the data for these men were compared with those for the rest of the subjects, no significant variations were found to exist in the blood volume and other hematologic characteristics, except that 1 of the complaining men had the highest values for the hemoglobin content and the hematocrit reading (21.10 Gm. and 60.6 per cent red cells, respectively).

INFLUENCE OF CHRONIC ANOXEMIA

Since the original observations of Bert,[1] Viault [2] and Müntz,[52] in the second half of the last century, numerous investigations have confirmed the existence of polycythemia in human beings and animals living at high altitudes. The review of the literature,[53] a summary of which

TABLE 14.—*Observations on Blood of Men Daily Exposed to a Low Barometric Pressure (Railroad Personnel)*

Subjects	Age, Years	Years of Exposure	Leukocytes, per Cu. Mm.	Leukocytic Differential Count, %							Blood Volume, Cc.; Gm. per Kg.			
				Stab Neutrophils	Segmented Neutrophils	Total Neutrophils	Eosinophils	Basophils	Monocytes	Lymphocytes	Blood	Plasma	Red Blood Cells	Hemoglobin
1	44	28	6,800	5	56	61	5	0	4	30	82.3	39.6	42.3	15.3
2	54	25	11,900	3	71	74	0	1	3	22	93.8	40.3	53.0	19.1
3	42	20	6,880	9	57	66	1	0	8	25	78.0	30.4	47.3	16.5
4	48	20	4,600	3	78	81	0	4	4	11	102.2	48.9	52.7	18.4
5	49	13	5,000	..	..	..	..	..	..	..	76.6	39.0	37.2	13.2
6	41	12	7,600	1	60	61	4	0	1	34	....	....	....	....
7	36	9	6,720	8	65.5	73.5	2.5	1	5.5	17.5	89.9	43.9	45.7	16.5
8	36	7	5,120	6	65	71	3.5	0	7.5	18	99.6	48.6	50.3	16.7
9	40	4	7,800	4	56	60	2	0	2	36	....	....	....	....
10	38	3	6,060	4	46	50	2.5	0	7.5	40	98.2	46.3	51.3	17.6
11	23	3	8,700	4	52	56	2	0	5	37	86.8	40.6	45.8	15.6
12	39	1	6,600	2	60	62	3	0	2	33	87.3	48.4	38.6	13.4
13	22	1	5,920	5	65	70	2.5	1	6	20.5	87.5	39.5	47.0	15.9
Mean ± P. E.			6,900 ± 356	4.5 ± 0.44	60.9 ± 1.67	65.4 ± 1.69	2.3 ± 0.28	0.6 ± 0.22	4.6 ± 0.44	27.0 ± 1.79	89.2 ± 1.81	42.3 ± 1.18	46.5 ± 1.14	16.2 ± 0.38
St. Dev. P. E.			1,830 ± 252	2.2 ± 0.31	8.2 ± 1.18	8.3 ± 1.19	1.4 ± 0.20	1.1 ± 0.15	2.2 ± 0.32	8.8 ± 1.26	8.1 ± 1.22	5.3 ± 0.79	5.1 ± 0.76	1.7 ± 0.25
Coef. of variation, %..			26.5	48.8	13.5	12.7	60.9	183.3	47.8	32.6	9.1	12.5	11.0	10.5

52. Müntz, A.: Compt. rend. Acad. d. sc. **112**:298, 1891.

53. (*a*) Fitzgerald, M. P.: Philos. Tr. Roy. Soc., London, s.B **203**:351, 1913. (*b*) Eggers, H.: München. med. Wchnschr. **73**:779, 1926. (*c*) Monge, C.; Heraud, C.; Encinas, E., and Hurtado, A.: Estudios fisiológicos sobre el hombre de los Andes, Lima, Facultad de Medicina, 1928. (*d*) Hurtado, A., and Guzmán Barrón, A.: Rev. med. peruana, Lima **1**:1, 1930. (*e*) Hurtado, A.: Am. J. Physiol. **100**:487, 1932. (*f*) Stammers, A. D.: J. Physiol. **78**:21P, 1933. (*g*) Liknaitzky, I.: Quart. J. Exper. Physiol. **24**:161, 1934. (*h*) Talbott, J. H., and Dill, D. B.: Am. J. M. Sc. **192**:626, 1936. (*i*) Andresen, M. I., and Mugrage, E. R.: Blood Cell Values for Normal Men and Women, Arch. Int. Med. **58**:136 (July) 1936. (*j*) Loewy, A., and Wittkower, E.: The Pathology of High Altitude, London, Oxford University Press, 1937. (*k*) Capdehourat, E. L., and others: Estudios sobre la biología del hombre de la altitud, Buenos Aires, Ministerio de Justicia e Instrucción Pública, 1937. (*l*) Barcroft and others.[3]

is given in table 15, indicates, however, a marked variability in the degree and other characteristics of the observed polycythemia; it is likely that this is related, at least in part, to the variable factors of altitude and the duration of exposure. We have made observations on three groups of men mines and in whom the degree of anoxemia was frequently abnormally accentuated owing to the pulmonary changes, and (*c*) men, also of Indian race, who had signs of loss of adaptation to the low pressure environment (chronic mountain sickness).

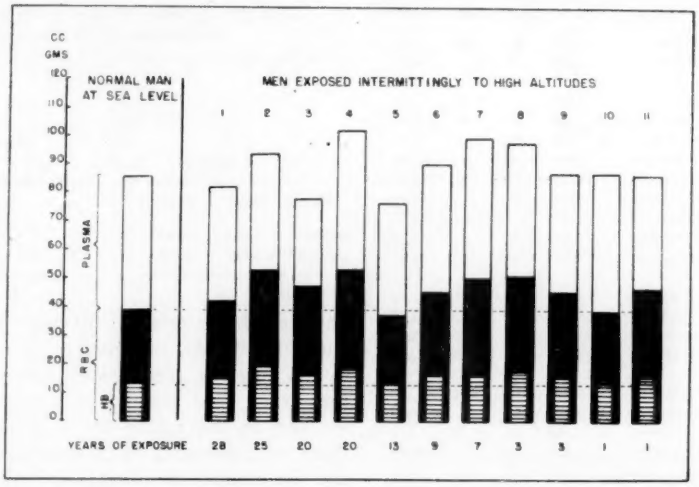
CC
GMS
NORMAL MAN
AT SEA LEVEL
MEN EXPOSED INTERMITTINGLY TO HIGH ALTITUDES
1 2 3 4 5 6 7 8 9 10 11
PLASMA
RBC
HB
YEARS OF EXPOSURE 28 25 20 20 13 9 7 3 3 1 1

Chart 10.—Blood volume (expressed in cubic centimeters and in grams per kilogram of body weight) of 11 men exposed daily to a low barometric pressure. The black zone represents red cell volume; the shaded zone, total hemoglobin; the white zone, plasma volume.

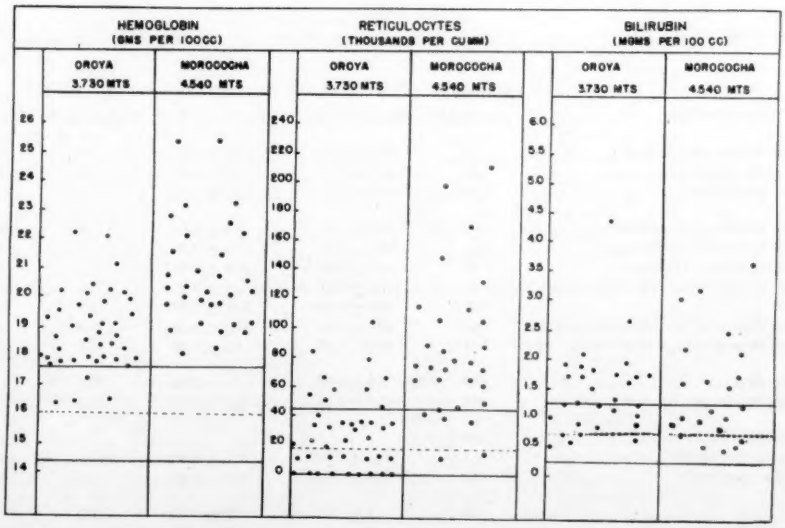

Chart 11.—Values for hemoglobin, reticulocytes and bilirubin in healthy native residents of Oroya (at 3,730 meters; 12,240 feet) and Morococha (at 4,540 meters; 14,900 feet). The zones between the horizontal lines represent the limits of variation observed in healthy adult men at sea level.

living permanently at high altitudes and subject, in consequence, to the constant influence of an anoxic condition: (*a*) healthy Indian natives; (*b*) Indian natives who had contracted pneumonoconiosis (silicosis) while working in the

Observations on Healthy Indian Natives.—Observations were made on a total of 72 Indian natives, 40 of them living and studied in Oroya, at an altitude of 3,730 meters (12,240 feet), with an average barometric pressure of 482 mm. of

TABLE 15.—*Previous Observations on Blood of Residents of High Altitudes (Summary of Literature [58])*

Investigators [58]	Place	Altitude, Meters	Number of Subjects	Red Blood Cells, Millions per Cu. Mm.	Hemoglobin, Gm. per 100 Cc.
Andresen, Mugrage..	Denver	1,520	40	5.42	16.54
Fitzgerald.....	Colorado	1,550	5	....	15.84
Kuendy, Loewy and others [64].....	Davos	1,580	..	6.55	.....
Liknaitzky.....	Johannesburg	1,750	60	5.99	(105.4%)
Stammers.....	Johannesburg	1,750	10	....	14.67
Fitzgerald.....	Colorado	1,830	6	....	15.02
Eggers.....	Mexico	2,250	22	7.25	(111.0%)
Fitzgerald.....	Colorado	2,370	10	....	16.40
Fitzgerald.....	Colorado	2,900	7	....	16.92
Fitzgerald.....	Colorado	3,080	9	....	17.16
Fitzgerald.....	Colorado	3,140	10	....	16.67
Hurtado, Guzman Barrón.....	Huancayo	3,200	10	5.82	.....
Fitzgerald.....	Colorado	3,450	22	....	17.46
Morales.....	La Paz	3,660	200	7.50	.....
Monge and others...	Oroya	3,730	20	6.88	.....
Capdehourat and others.....	Catavi	3,750	11	6.31	.....
Fitzgerald.....	Colorado	4,300	16	....	18.05
Barcroft and others	C. Pasco	4,330	15	7.05	.....
Barcroft and others	C. Pasco	4,330	10	....	18.85
Hurtado.....	Morococha	4,540	100	6.66	15.93
Talbott and Dill.....	Quilchua	5,340	6	7.37	22.86

mercury, and 32 residents of Morococha, where they were investigated, at an elevation of 4,540 meters (14,900 feet), with a mean barometric pressure of 446 mm. of mercury. The ages of these men varied between 19 and 48 years, but only 1 of them was in the fifth decade. Their physical characteristics (average height, weight and surface area, 1.57 meters, 56.6 Kg. and 1.57 square meters, respectively) corresponded to their race, and none gave a history of having worked in the mines for a prolonged time, a point of importance in these zones on account of the frequency of pneumonoconiosis. All the observed men had been born either at the places,

TABLE 16—*Degree of Anoxemia in Residents of Oroya and Morococha*

Place	Altitude, Meters	Altitude, Feet	Average Barometric Pressure, Mm. Hg	No. of Subjects	Arterial Oxygen Saturation, per Cent: Mean ± P. E.	Arterial Oxygen Saturation, per Cent: Extreme Variations
Oroya	3,730	12,240	482	15	87.6 ± 0.27	84.4 - 90.2
Morococha	4,540	14,900	446	18	81.4 ± 0.45	75.2 - 86.2

TABLE 17.—*Observations on Blood of Healthy Men Residents of Oroya (at an Altitude of 3,730 Meters; 12,240 Feet)*

Determinations	Number of Subjects	Mean ± P. E.	St. Dev. ± P. E.	Coefficient of Variation, %	Extreme Variations
Red blood cells (millions per cu. mm.).....	40	5.67 ± 0.04	0.39 ± 0.03	6.9	4.70 - 6.28
Hematocrit (red cells, per cent).....	40	54.1 ± 0.41	3.9 ± 0.21	7.2	47.8 - 65.4
Hemoglobin (Gm. per 100 cc.).....	40	18.82 ± 0.15	1.46 ± 0.15	7.7	16.40 - 22.05
Corpuscular mean volume (cu. microns).....	40	95.2 ± 0.58	5.5 ± 0.41	5.8	84.2 - 113.1
Corpuscular mean diameter (microns)	40	7.88 ± 0.03	0.24 ± 0.02	3.0	7.42 - 8.52
Corpuscular mean thickness (microns)	40	1.97 ± 0.02	0.17 ± 0.01	8.6	1.48 - 2.22
Corpuscular mean surface area (sq. microns).....	40	145.8 ± 0.47	5.1 ± 0.33	3.5	133.6 - 158.0
Spherocytic index	40	0.25 ± 0.002	0.03 ± 0.001	12.0	0.17 - 0.30
Corpuscular mean hemoglobin (micromicrograms).	40	33.0 ± 0.25	2.4 ± 0.18	8.0	28.7 - 38.4
Corpuscular mean hemoglobin concentration (%).	40	34.8 ± 0.10	0.9 ± 0.06	3.0	32.1 - 36.6
Reticulocytes (per cent)	40	0.8 ± 0.06	0.5 ± 0.04	62.5	0 - 2.2
Reticulocytes (thousands per cu. mm.).....	40	38.0 ± 2.90	24.3 ± 2.05	63.9	0 - 105.8
Red blood cell fragility.....	30				
Initial hemolysis (per cent).....	..	0.46 ± 0.001	0.01 ± 0.001	2.2	0.44 - 0.50
Total hemolysis (per cent).....	..	0.38 ± 0.002	0.02 ± 0.001	5.2	0.34 - 0.42
Blood viscosity	30	8.4 ± 0.21	1.7 ± 0.15	20.2	5.8 - 15.2
Bilirubin, total (mg. per 100 cc.).....	30	1.47 ± 0.09	0.71 ± 0.06	48.3	0.56 - 4.38
Leukocytes (per cu. mm.).....	40	6,500 ± 155	1,258 ± 109	19.2	3,400 - 9,600
Differential leukocytic count.....	40				
Neutrophils, stab (per cent).....	..	5.0 ± 0.30	2.8 ± 0.21	56.0	0 - 2
Neutrophils, segmented (per cent).....	..	52.8 ± 1.13	10.6 ± 0.79	20.1	32 - 79
Neutrophils, total (per cent).....	..	57.9 ± 1.13	10.6 ± 0.79	18.3	37 - 86
Eosinophils (per cent).....	..	2.5 ± 0.22	2.1 ± 0.15	84.0	0 - 10
Basophils (per cent).....	..	0.1 ± 0.007	0.7 ± 0.005	700.0	0 - 3
Monocytes (per cent).....	..	4.9 ± 0.24	2.3 ± 0.16	46.9	0 - 9
Lymphocytes (per cent).....	..	34.8 ± 1.02	9.6 ± 0.72	27.6	11 - 54

TABLE 18.—*Observations on Blood of Healthy Men Residents of Morococha (at an Altitude of 4,540 Meters; 14,900 Feet)*

Determinations	Number of Subjects	Mean ± P. E.	St. Dev. ± P. E.	Coefficient of Variation, %	Extreme Variations
Red blood cells (millions per cu. mm.)	32	6.15 ± 0.07	0.57 ± 0.05	9.3	4.97 - 7.59
Hematocrit (red cells, per cent)	32	59.9 ± 0.66	5.6 ± 0.47	9.3	52.2 - 76.2
Hemoglobin (Gm. per 100 cc.)	32	20.76 ± 0.20	1.71 ± 0.14	8.2	18.10 - 25.40
Corpuscular mean volume (cu. microns)	32	97.5 ± 0.75	6.3 ± 0.53	6.5	85.5 - 106.8
Corpuscular mean diameter (microns)	32	7.74 ± 0.01	0.11 ± 0.01	1.4	7.31 - 8.01
Corpuscular mean thickness (microns)	32	2.08 ± 0.02	0.16 ± 0.01	7.7	1.70 - 2.41
Corpuscular mean surface area (sq. microns)	32	145.0 ± 1.18	9.9 ± 0.83	6.8	133.6 - 158.6
Spherocytic index	32	0.27 ± 0.002	0.2 ± 0.001	7.4	0.21 - 0.32
Corpuscular mean hemoglobin (micromicrograms)	32	33.9 ± 0.29	2.5 ± 0.21	7.3	28.4 - 37.4
Corpuscular mean hemoglobin concentration (%)	32	34.7 ± 0.12	1.0 ± 0.08	2.9	33.2 - 37.4
Reticulocytes (per cent)	32	1.5 ± 0.07	0.6 ± 0.05	40.0	0.2 3.3
Reticulocytes (thousands per cu. mm.)	32	83.4 ± 5.35	44.9 ± 3.79	53.8	10.8 - 210.5
Bilirubin, total (mg. per 100 cc.)	20	1.56 ± 0.19	1.22 ± 0.13	78.2	0.45 - 4.91
Bilirubin, direct (mg. per 100 cc.)	20	0.46 ± 0.03	0.17 ± 0.02	36.9	0.16 - 0.86
Bilirubin, indirect (mg. per 100 cc.)	20	1.10 ± 0.17	1.10 ± 0.12	100.0	0.23 - 4.32
Leukocytes (per cu. mm.)	32	6,900 ± 144	1,212 ± 102	17.6	4,700 - 10,900
Differential leukocytic count	32				
Neutrophils, stab (per cent)	..	3.0 ± 0.22	1.8 ± 0.15	60.0	0 - 6
Neutrophils, segmented (per cent)	..	49.9 ± 1.34	10.9 ± 0.94	21.0	25 - 71
Neutrophils, total (per cent)	..	52.7 ± 1.33	10.8 ± 0.94	20.5	27 - 71
Eosinophils (per cent)	..	2.2 ± 0.15	1.2 ± 0.10	54.5	0 - 5
Basophils (per cent)	..	0.2 ± 0.02	0.5 ± 0.01	250.0	0 - 2
Monocytes (per cent)	..	5.5 ± 0.34	2.8 ± 0.24	50.9	1 - 13
Lymphocytes (per cent)	..	39.4 ± 1.29	10.5 ± 0.91	26.6	21 - 62

TABLE 19.—*Determinations of Blood Volume of Healthy Native Male Residents at High Altitudes*

Determinations	Mean ± P. E.	St. Dev. ± P. E.	Coefficient of Variation, %	Extreme Variations
A. With Brilliant Vital Red (30 Subjects)				
1. In Oroya (at 3,730 Meters; 12,240 Feet)				
Blood volume (liters)	6.15 ± 0.12	1.03 ± 0.09	16.7	4.26 - 9.05
Blood volume (cc. per Kg.)	108.7 ± 1.69	13.7 ± 1.19	12.6	79.6 - 149.6
Plasma volume (liters)	2.76 ± 0.05	0.44 ± 0.03	15.9	1.94 - 3.72
Plasma volume (cc. per Kg.)	48.9 ± 0.87	7.1 ± 0.61	14.5	35.9 - 65.9
Red cell volume (liters)	3.36 ± 0.12	0.98 ± 0.08	29.1	2.22 - 5.67
Red cell volume (cc. per Kg.)	59.7 ± 1.07	8.7 ± 0.76	14.6	41.5 - 93.2
Total hemoglobin (Gm.)	1,150 ± 28	226 ± 19	19.6	758 - 1,819
Total hemoglobin (Gm. per Kg.)	20.7 ± 0.40	3.3 ± 0.28	15.9	14.2 - 30.1
2. In Morococha (at 4,540 Meters; 14,900 Feet)				
A. With Brilliant Vital Red (6 Subjects)				
Blood volume (liters)	6.98 ± 0.50	1.50 ± 0.36	21.5	5.09 - 10.14
Blood volume (cc. per Kg.)	120.8 ± 4.08	13.5 ± 1.94	11.2	113.5 - 145.9
Plasma volume (liters)	2.65 ± 0.30	0.44 ± 0.10	16.6	2.00 - 3.50
Plasma volume (cc. per Kg.)	46.1 ± 1.27	4.2 ± 0.90	9.1	40.8 - 52.1
Red cell volume (liters)	4.29 ± 0.38	1.14 ± 0.27	26.6	3.03 - 6.59
Red cell volume (cc. per Kg.)	74.1 ± 3.86	12.8 ± 2.73	17.2	54.1 - 94.8
Total hemoglobin (Gm.)	1,464 ± 123	364 ± 87	24.9	1,084 - 2,185
Total hemoglobin (Gm. per Kg.)	25.2 ± 1.17	3.9 ± 0.81	15.4	19.3 - 21.4
B. With Evans Blue (11 Subjects)				
Blood volume (liters)	5.89 ± 0.16	0.78 ± 0.11	13.2	5.01 - 7.58
Blood volume (cc. per Kg.)	100.3 ± 2.30	10.8 ± 1.62	10.8	86.4 - 126.4
Plasma volume (liters)	2.14 ± 0.06	0.27 ± 0.04	12.6	1.66 - 2.59
Plasma volume (cc. per Kg.)	36.2 ± 0.55	2.6 ± 0.39	7.2	32.8 - 40.8
Red cell volume (liters)	3.73 ± 0.15	0.71 ± 0.11	19.0	2.88 - 5.56
Red cell volume (cc. per Kg.)	64.1 ± 2.39	11.2 ± 1.69	17.5	49.7 - 92.7
Total hemoglobin (Gm.)	1,293 ± 54.1	254 ± 38.2	19.6	997 - 1,926
Total hemoglobin (Gm. per Kg.)	22.0 ± 0.87	4.1 ± 0.61	18.6	17.2 - 32.1

or nearby, where they were investigated or at altitudes over 3,050 meters (10,000 feet), in the latter case with a long residence in Oroya or Morococha. For 15 residents of Oroya and 18 of Morococha arterial blood was obtained and analyzed for its oxygen saturation in order to determine the degree of anoxemia to which the hematologic characteristics corresponded. Tables 16, 17, 18 and 19 contain the results obtained in all the studies made for these series of subjects. In Oroya, the lower locality, where the mean arterial oxygen saturation was found to be 87.6 ± 0.27 per cent, with variations between 84.4 and 90.2 per cent, the mean hemoglobin content was 18.82 ± 0.15 Gm. per hundred cubic

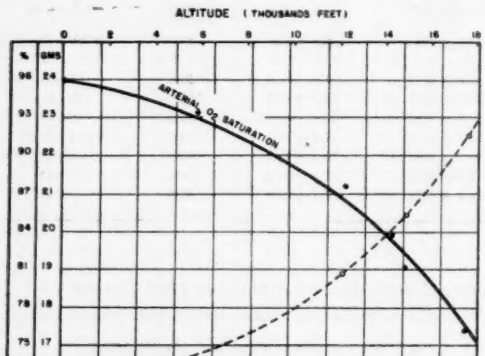

Chart 12.—Relationship between the mean arterial oxygen saturation (per cent) and the mean hemoglobin content (grams per hundred cubic centimeters) in healthy male residents at different altitudes. The points used in the construction of the curves correspond to our observations and those made by Barcroft and others,[3] Stammers,[53f] Talbott and Dill[53h] and Andresen and Mugrage.[53i]

centimeters; in 36 men (90 per cent) the concentration exceeded 17.50 Gm., with a maximal value of 22.05 Gm., and in only 4 subjects (10 per cent) it fell within the limits of variations seen at sea level (chart 11). The hematocrit reading also showed proportional increases; in 35 men (87.5 per cent) it was higher than 50.0 per cent red cells, with a maximal value of 65.4 per cent. In Morococha, the higher place, with a mean arterial oxygen saturation of 81.4 ± 0.45 per cent and variations between 75.2 and 86.2 per cent, the increases in the hemoglobin and hematocrit values were greater, as shown by the mean values of 20.76 ± 0.20 Gm. and 59.9 ± 0.66 red cells per cent.[54] All subjects had hemoglobin and hematocrit values over 17.50 Gm. and 50.0 per cent, respectively; in 2 cases the high figures of 25.40 Gm. and 76.2 per cent were obtained.

The graphic correlation of the mean values of arterial oxygen saturation and the hemoglobin concentration corresponding to residents at sea level and at different altitudes obtained in previous investigations[55] and by us, in which the hemoglobin has been calculated from the accurate determination of the oxygen-binding capacity of

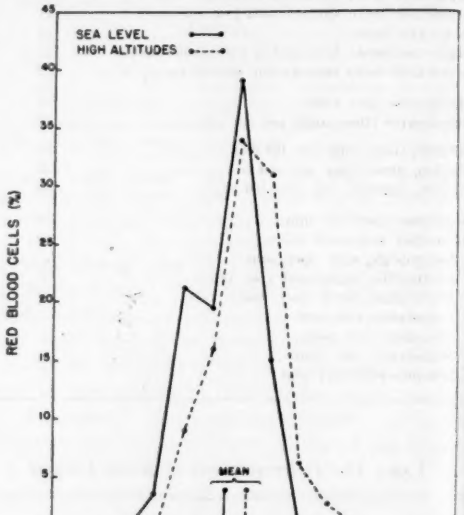

Chart 13.—Diameter of the red blood cells at sea level and at high altitudes. The solid line represents measurements made for 130 healthy men living at sea level (a total of 39,400 cells measured); the interrupted line, measurements made for 72 healthy male residents of Oroya (at 3,740 meters; 12,240 feet) and Morococha (4,540 meters; 14,900 feet) (a total of 17,100 cells measured).

the blood, shows a striking inverse relationship between the degree of arterial oxygen saturation and the level of hemoglobin in the circulating blood (chart 12).

The morphologic characteristics of the red blood cells varied in some respects from those observed in healthy residents at sea level (table 20). The circulating erythrocyte at high alti-

54. The mean value of 20.76 ± 0.20 Gm. of hemoglobin is considerably higher than 15.93 ± 0.20 Gm., a mean obtained by one of us (A. H.)[53e] in 1932 in observations made on healthy men living in the same locality. The reason for this discrepancy was found in a later investigation to be due to the water used for the dilution of the blood sample in the Sahli hemoglobinometer, a technic employed in the 1932 study; after addition of the water available in this place the color of the hematin solution faded rapidly, giving, in consequence, abnormal low values.

55. Barcroft and others.[3] Stammers.[53f] Talbott and Dill.[53h] Andresen and Mugrage.[53i]

tudes has a larger size, as shown by an increased mean volume, diameter and surface area; the Price-Jones curves corresponding to the measurement of the diameter of several thousands of red cells at sea level and at high altitudes (chart 13) revealed a displacement to the right in the latter places, where 40.8 per cent of the cells had a diameter above 8.00 microns, as compared with 16.3 per cent at sea level; on the other hand, a diameter of less than 7.00 microns was observed in 24.9 per cent of the red cells of subjects studied at sea level, in contrast with 9.6 per cent at high altitudes. The tendency to a slight macrocytosis at high altitudes has been observed in previous investigations [52]; mean corpuscular volumes of 96.2 and 94.1 cubic microns have been found for the native residents of Morococha and

TABLE 20.—*Morphologic Characteristics of the Red Blood Cells of Healthy Men (Residents) at Different Altitudes*

	Lima	Oroya	Morococha
Altitude	Sea level	3,730 meters	4,540 meters
Number of subjects	175	40	32
	Mean ± P. E.		
Corpuscular mean volume (cu. microns)	91.3 ± 0.23	95.2 ± 0.58	97.5 ± 0.75
Corpuscular mean diameter (microns)	7.48 ± 0.01	7.88 ± 0.03	7.74 ± 0.01
Corpuscular mean thickness (microns)	2.09 ± 0.01	1.97 ± 0.02	2.08 ± 0.02
Corpuscular mean surface area (sq. microns)	137 ± 0.22	146 ± 0.47	145 ± 1.18
Spherocytic index	0.28 ± 0.01	0.25 ± 0.01	0.25 ± 0.01
Corpuscular mean hemoglobin (micromicrograms)	31.2 ± 0.09	33.0 ± 0.25	33.9 ± 0.29
Corpuscular mean hemoglobin concentration (%)	34.1 ± 0.07	34.8 ± 0.10	34.7 ± 0.12

Quilchua (at 4,540 and 5,340 meters, respectively). Talbott [56] reported an increase in the size of the red blood cells in the members of the Chilean High Altitudes Expedition after a prolonged residence. The high red cell count associated with the moderate increase in the cell volume at high altitudes makes the latter characteristic more significant, because at sea level a greater number of red cells per cubic millimeter is accompanied by a decreased cell size (table 4). The hemoglobin content of the red cells was found to be elevated in the residents of high altitudes as compared with the observations made at sea level, but the increase corresponded to their larger size, as shown by the corpuscular hemoglobin concentration (per cent), which was practically unchanged at all altitudes.

It has been postulated by some investigators [57] that the hemoglobin is distributed on the surface of the red cells; a statistical study of our observations on healthy men living at sea level and at high altitudes (which include a total of 130 and 72 men, respectively) fails, however, to support such a view. The coefficients of correlation between mean corpuscular volume and mean corpuscular hemoglobin were found to be + 0.7791 ± 0.0232 and + 0.8636 ± 0.0202 for the series of subjects studied at sea level and at high altitudes, respectively, while the corresponding coefficients between mean corpuscular surface area and mean corpuscular hemoglobin were + 0.5272 ± 0.0427 and + 0.4503 ± 0.0633.

The mean values for the reticulocytes, in per cent and per cubic millimeter, were higher than the corresponding ones at sea level, and the increase was especially marked at the higher altitude (Morococha), where the large majority of the natives showed values well above the upper sea level limit of variation (chart 11); the highest observed value was 3.3 per cent. Barcroft and his collaborators [3] found that 5 native residents of Cerro de Pasco (4,330 meters; 14,200 feet of altitude) had reticulated counts between 1.2 and 2.0 per cent, with an average of 1.5 per cent, and that 4 American residents had counts between 1.3 and 1.5 per cent. At the extremely high elevation of 5,340 meters (17,500 feet) Talbott and Dill [53h] observed counts of 0.4, 0.6 and 3.4 per cent in the blood of 3 residents. No nucleated red cells in the peripheral blood have been observed in previous investigations or by us in residents of high altitudes.

A definite rise was observed in the serum bilirubin, which confirmed previous but less complete observations.[53c, e] The mean values of 1.47 ± 0.09 and 1.56 ± 0.19 mg. per hundred cubic centimeters, obtained in Oroya and in Morococha, respectively, indicated approximately 100 per cent increase over the mean normal value at sea level, and in the majority of the subjects the pigment was present in a concentration exceeding 1.00 mg. per hundred cubic centimeters (chart 11). At the higher altitude (4,540 meters; 14,900 feet) we determined the bilirubin in its total and fractionated forms in 20 natives; it was found that the increase affected almost exclusively the indirectly determined pigment; the ratio direct: total bilirubin had an average value of 29.5 per cent, in contrast with 51.4 per cent observed at sea level.

The bilirubin-excretory function of the liver was investigated in 4 native residents of Moro-

56. Talbott, J. H.: Folia haemat. **55**:23, 1936.

57. Bürker, K.: Arch. f. Physiol. **195**:516, 1922. Brinkman, R., and Szent-György, A.: J. Physiol. **58**: 204, 1923. Rabinowitch, I. M., and Strean, G.: Hemoglobin Content of Red Blood Cells in Relation to Their Surface Area, Arch. Int. Med. **34**:124 (July) 1924.

cocha by injecting 50 mg. of bilirubin and determining its concentration in the blood three hours after the injection (method of von Bergman, quoted by Dameshek and Singer [23]); the same men were brought down to sea level ten days later, where the test was repeated twenty to thirty-six hours after arrival. For comparative purposes the same test was performed for 4 residents of sea level and repeated immediately after their arrival to Morococha. The results obtained in the sixteen tests are given in chart 14. The 4 natives studied in Morococha, all with an initial bilirubin concentration above 1.00 mg. per hundred cubic centimeters, showed a higher degree of retention of the injected bilirubin than the men studied at sea level; in 2 of them the retention was marked (62.9 and 86.7 per cent); when brought down to sea level all showed an increase in the basal value for bilirubin, but, on the other hand, the excretion of the injected pigment was accomplished in a more complete way in 3 of them; in 1 it was practically unchanged. No significant alterations were observed in the group of 4 men studied first at sea level and later on arrival at high altitude in regard to the basal pigment and the degree of retention of the injected bilirubin.

The viscosity of the blood of 30 native residents of Oroya (3,730 meters; 12,240 feet) had a mean value of 8.4 ± 0.21 with a standard deviation of 1.7, figures which are distinctly higher than the corresponding ones, of 4.64 ± 0.025 and 0.86, obtained by Nygaard, Wilder and Berkson [58] for 503 subjects studied at sea level. Holbrook and Watson [59] found, also at sea level, an average value of 5.08, with variations between 4.0 and 6.0, in 97 normal subjects.

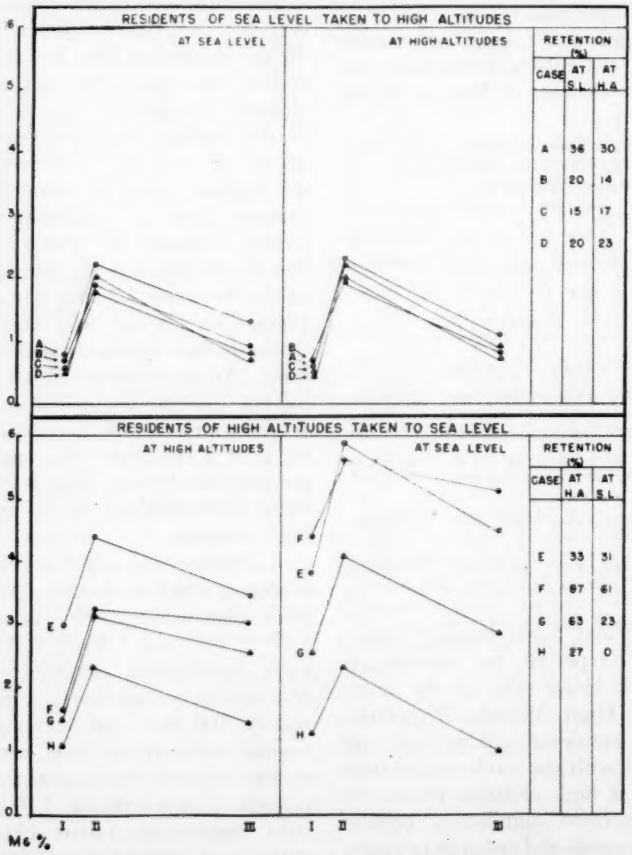

Chart 14.—Results of bilirubin excretion tests of healthy men at sea level and at high altitudes. *I*, basal serum bilirubin; *II*, serum bilirubin five minutes after an intravenous injection of 50 mg.; *III*, serum bilirubin three hours after the injection. Notice the increase in the basal bilirubin which occurs when men living at high altitudes are brought down to sea level.

58. Nygaard, K. K.; Wilder, M., and Berkson, J.: Am. J. Physiol. **114**:128, 1935.

59. Holbrook, A. A., and Watson, B. M.: Am. J. M. Sc. **198**:750, 1939.

The fragility of the red blood cells to hypotonic solutions of sodium chloride was found to be essentially normal in the subjects investigated in Oroya. The mean values, of 0.46 ± 0.001 and 0.38 ± 0.002 per cent, for initial and total hemolysis, respectively, were the same as those obtained at sea level. Only in 2 subjects the values obtained for initial hemolysis were slightly higher than the upper limit observed at sea level, which showed some evidence of increased fragility.

The number of leukocytes per cubic millimeter did not show any significant alteration in the native residents of high altitudes. The mean observed values in Oroya and Morococha were almost identical with the one observed at sea level; in only 1 subject at the higher altitude, a slight leukocytosis (10,900 white cells per cubic millimeter) was observed. On the other hand, the differential leukocyte count when compared with the sea level values showed a mean percentage increase in the lymphocytes, more marked the higher the altitude, with a corresponding decrease in the segmented neutrophils and eosinophils and, to a lesser degree, in the monocytes; the mean values of the stab neutrophils and basophils did not show any appreciable change. This relative lymphocytosis has been observed in previous investigations made at high altitudes; these have been summarized by Loewy and Wittkower.[58j] In this respect it is interesting to mention that the variation in the differential leukocyte count has been found to exist in residents of elevations below 2,000 meters (6,560 feet).[60]

The circulating blood volume was investigated for 47 healthy Indian natives, 30 of them residents of Oroya (at 3,730 meters; 12,240 feet) and 17 residents of Morococha (at 4,540 meters; 14,900 feet). In the latter place 11 of the determinations were carried out by means of injection of the dye Evans' blue (method of Gibson and Evans [15]); all the others were made with the dye brilliant vital red.[37] The results obtained in both places agreed in the finding of an increased total cell volume in all subjects investigated, but the degree of the increase was not similar in the two methods (table 19). In Oroya, the lower place, the cell volume, expressed in cubic centimeters per kilogram of body weight, showed a mean increase of 59.7 per cent over the corresponding sea level value; in Morococha, the higher altitude, the mean increase was 90.9 per cent with the brilliant vital red method and 65.2 per cent with the Evans' blue. The plasma volume, in cubic centimeters per kilogram of body weight, was found to be practically unchanged at both altitudes, according to the determinations made with brilliant vital red, but had an average decrease of 23.1 per cent in Morococha, in those men in whom the Evans' blue dye was employed.

The discrepancy between the results obtained by the two dye methods cannot be strictly attributed to their different technical characteristics; the small number of subjects studied in each series may be an important factor in explaining the higher cell volume observed with the brilliant vital red. In a recent observation, not included in this paper, a determination of the blood volume made for 1 healthy native resident of Morococha by means of the dye Evans' blue gave the values of 165.2, 32.0 and 132.3 cc. of blood, plasma and red cells, respectively, per kilogram of body weight. Another indication of the possible accuracy of the results observed with brilliant vital red is the high correlation coefficient, of + 0.8008 ± 0.0269, obtained between the hematocrit level and the total red cell volume of 79 subjects studied at high altitudes with this dye method (including healthy natives and persons with pathologic conditions, the latter to be discussed in the next chapter). An analogous correlation has been found to exist in cases of polycythemia vera,[61] in the study of which Evans' blue dye was employed, and in dogs investigated by means of radioactive iron.[62]

Taking into consideration the objections which have been raised in regard to the use of the hematocrit in the determination of the total blood volume and the recent criticisms [63] concerning the accuracy of the linear extrapolation for the calculation of the plasma volume with Evans' blue dye, it seems appropriate at the present time to stress the value of comparative studies made with similar technics rather than the strict significance of absolute figures obtained by a given method. In reviewing the importance of determinations of blood volume Keith [64] has recently remarked that "the ideal method of estimating plasma and blood volume has still to be discovered."

The existence of a polycythemic normovolemia or hypervolemia in residents of high altitudes has been demonstrated in previous investigations made at moderate elevations. Lippmann,[65] in 1926, obtained average values of 87.3, 40.2 and 47.1 cc. of blood, plasma and red cells per kilogram of body weight in 4 men living in Davos, at an altitude of 1,590 meters (5,000 feet); Lozoya Solís,[66] in 1936, determined the blood volume of 53 healthy men living in the city of Mexico, located at an altitude of 2,260 meters (7,140 feet) and found mean values of 92.4 and

60. Ruppanner, E.: Schweiz. med. Wchnschr. **50**: 105, 1920. Stammers, A. D.: J. Physiol. **78**:335, 1933. Peterson, R. F., and Peterson, W. G.: J. Lab. & Clin. Med. **20**:723, 1935. Fitzgerald.[58a]

61. Gibson, J. G.: Ann. Int. Med. **14**:2014, 1941.

62. Hahn, P. H., and Bale, W. F.: Am. J. Physiol. **136**:314, 1942.

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50.7 cc. of blood and red cells per kilogram of body weight, which he considered to represent increases of 8.7 and 12.7 per cent, respectively, over the sea level values. The plasma volume was unchanged. The correlation of our findings with those obtained in these investigations seems to indicate that there is an inverse relationship between the volume of circulating red cells, expressed in cubic centimeters per kilogram of body weight, and the arterial oxygen saturation. The higher the altitude and the degree of anoxemia, the more marked is the increase in the cell volume, while the plasma volume remains unchanged or decreases.

The polycythemia associated with constant exposure to a low barometric pressure has also been long since verified in animals living at high altitudes.[67] Izquierdo [67a] cited the investigations of Vergara Lopez, who in studies made in the city of Mexico in 1899 found an increase of red blood cells in guinea pigs. Hall, Dill and Barrón [67c] observed an increased amount of hemoglobin in the blood of sheep and rabbits living at altitudes over 3,050 meters (10,000 feet); these investigators made the interesting observation that in the llama, a native animal of the high Andean plateau, an increasing level of altitude corresponded to a decreasing amount of hemoglobin in the blood. Rotta [67d] has observed in dogs living in Morococha (at 4,540 meters; 14,900 feet) changes in the blood volume parallel to those found by us in the Indian native residents of that place; in determinations made in 9 dogs he found an increase of 44.5 per cent in the red cell volume, while the plasma volume was almost identical to the one observed in the comparative studies made at sea level (table 21).

TABLE 21.—*Determinations of Blood Volume* of Dogs at Sea Level and in Morococha (at 4,540 Meters; 14,900 Feet); Data Taken from Rotta* [67d]

	Sea Level	Morococha
Number of dogs	8	9
Average weight (Kg.)	9.0	7.6
	Mean ± P. E.	
Blood volume (cc. per Kg.)	80.7 ± 1.04	96.0 ± 2.62
Plasma volume (cc. per Kg.)	45.9 ± 0.94	45.7 ± 1.50
Red cell volume (cc. per Kg.)	34.6 ± 1.14	50.0 ± 2.22
Hemoglobin (Gm. per Kg.)	11.0 ± 0.27	15.4 ± 0.31

* With dye brilliant vital red.

67. (*a*) Izquierdo, J. J.: Compt. rend. Soc. de biol. **87**:1195, 1922. (*b*) Mulligan, R. M.: Am. J. Physiol. **133**:394, 1941. (*c*) Hall, F. G.; Dill, D. B., and Barrón, E. S. G.: J. Cell. & Comp. Physiol. **8**:301, 1936. (*d*) Rotta, A.: La circulación en las grandes alturas, Lima, Facultad de Medicina, 1938.

Observations in Cases of Pneumonoconiosis (Silicosis).—Studies were made in Oroya (at an altitude of 3,730 meters [12,240 feet]) on 82 persons who had silicosis acquired while they were working in mines located at high altitudes. Owing to the anatomic alterations in the lungs these patients often showed an abnormal accentuation of the arterial oxygen unsaturation present in some degree in all dwellers of high altitudes. It was, in consequence, interesting to investigate the effects of this increased degree of anoxemia on the hematologic characteristics of the circulating blood.

All the silicotic persons were Indian natives born and living at high altitudes; their ages varied between 25 and 56 years. Their physical characteristics corresponded to their race, and, with few exceptions, undernourishment was not revealed by the clinical examination. The diagnosis of silicosis was made with consideration of the occupational history (exposure to siliceous dust while working in the mines) and the findings in the roentgenogram of the chest. The pulmonary lesions fluctuated from discrete nodulations to large areas of confluent fibrosis. No person with tubercle bacilli in the sputum or with an obvious associated tuberculosis has been included in this series. In each case, in addition to and simultaneous with the taking of the venous blood sample on which the different hematologic studies were carried out, an arterial puncture was made and the blood taken analyzed for its carbon dioxide content and oxygen saturation. The latter varied between 43.3 and 90.8 per cent. Forty of the men showed a saturation of 84.0 per cent or higher, corresponding to that found in the healthy residents of the altitude where the investigations were made; in 33 it varied between 72.0 and 83.9 per cent; in 5 it was found to fluctuate between 60.0 and 71.9 per cent; and, finally, in the remaining group of 4 the arterial oxygen saturation was less than 60.0 per cent, the lowest value being 43.3 per cent. The last two groups included 4 silicotic men with obvious clinical signs of an associated circulatory insufficiency. The results obtained in the different investigations on the blood are given, in relation to the degree of anoxemia, in tables 22 and 23 and presented graphically in charts 15 and 16.

Men with silicosis in whom no accentuation of the degree of anoxemia was caused by the pulmonary lesions presented hematologic characteristics similar to those observed in the healthy

residents of the altitudes where they lived.[68] On the other hand, silicotic men with an abnormal and more pronounced unsaturation of the arterial blood showed definite rises in the concentration of red blood cells and hemoglobin, which were, up to a certain limit, greater the higher the degree of anoxemia. The 3 highest hemoglobin and hematocrit values observed were 26.89, 27.47, 28.68 Gm. per hundred cubic centimeters and 81.1, 81.2 and 82.3 per cent red cells, respectively. The determination of the blood volume of the respective subjects revealed, similarly, a marked increase in the cell volume, also in proportion to the degree of anoxemia, accompanied by a decreased plasma volume. In 11, or 25 per cent of the cases studied, the blood volume was higher than 150 cc. per kilogram of body weight, and in 13 cases, or 29.5 per cent, the red cell volume, similarly expressed, was higher than 100 cc. In a case of advanced silicosis, studied subsequently and not included in this series, a determination of blood volume made with Evans blue dye gave the following results: 150.2, 48.2 and 101.2 cc. of blood, plasma and red cells, respectively, per kilogram of body weight.

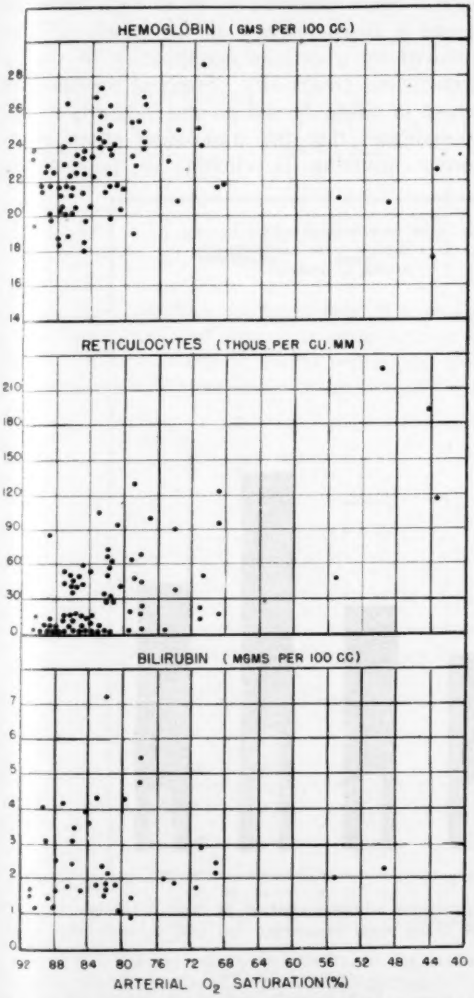

Chart 15.—Relation between values for hemoglobin, reticulocytes and bilirubin and the degree of arterial oxygen saturation in persons with silicosis studied at high altitudes.

These men with silicosis, all studied at the same altitude and showing great variability in the degree of anoxemia and in the level of polycythemia, offered a good opportunity to investigate the possible influence of other factors, in addition to the arterial oxygen insaturation, on the hematologic characteristics. In regard to the carbon dioxide content of the arterial blood, an increase of which has been cited as capable of eliciting a polycythemic response,[69] a correlation coefficient of -0.3275 ± 0.0686 was obtained between the arterial content of this gas (in volume per cent) and of hemoglobin (grams per hundred cubic centimeters), in contrast to the one of -0.4265 ± 0.0629 obtained between the arterial oxygen saturation (in per cent) and the hemoglobin (similarly expressed). Both correlations were linear (no significant difference with the correlation ratios) and independent of each other (the partial correlation coefficients had the same values).

The possibility of an unequal distribution of red blood cells in the vascular system, a factor which in the past has been thought to explain, at least in part, the polycythemia of high altitudes,[70] also has been investigated: for 44 subjects (some of whom were healthy men) red blood cell counts were made on samples of capillary and venous blood taken simultaneously; the mean observed counts were 6.30 ± 0.09 and 6.21 ± 0.10 millions per cubic millimeter, respectively; in 66 per cent of the cases the capillary count was higher than the venous, the average difference being 0.24 millions. In 15 cases of silicosis the red cell count was determined on samples of capillary, venous and arterial blood taken simultaneously; the mean values (millions of red cells per cubic millimeter) corresponding to these series of determinations were found to be as follows: capillary blood, 7.52 ± 0.22; venous blood, 7.33 ± 0.20; arterial blood, 7.19 ± 0.21. The differences between these mean values have no statistical significance. Finally, in 19 cases of silicosis a hematocrit determination was made on venous and on arterial blood, also taken simultaneously; the mean observed values were 69.6 ± 1.15 and 69.7 ± 1.14 per cent red cells, respectively. These results show that the degree of polycythemia was essentially similar

68. All the men with silicosis lived at altitudes of about 4,200 to 4,500 meters (13,800 to 14,800 feet), where the mines were located, but the study was made one or two days after their arrival at Oroya, at an altitude of 3,730 meters (12,240 feet). Thus, the arterial oxygen saturation found in the investigations corresponded to the latter altitude, while the hematologic characteristics were related to the influence of the higher altitudes where they lived.

69. Jordan, H. E., and Speidel, C. C.: J. Exper. Med. **40**:1, 1924.

70. Campbell, W. A., and Hoagland, H. W.: Am. J. M. Sc. **122**:654, 1901. Foà, C.: Lab. sc. internat. du Mont Rosa, Turin **1**:15, 1904. Goyda, T.: Chem. Zentralbl. **1**:672, 1911.

in the different sections of the vascular system, with a slight tendency to be higher in the capillary circuit.

The various morphologic characteristics of the circulating erythrocytes were similar to those found in the group of healthy natives; a slight macrocytosis and a normal corpuscular hemoglobin concentration were the essential findings. Fewer reticulocytes were observed in these subjects; this was perhaps related to the fact that they were studied at an altitude lower than their habitual place of residence. The serum bilirubin was elevated in almost all the subjects; in 12 men, or 27.3 per cent of the total, the concentration exceeded 3.00 mg. per hundred cubic centimeters, and the highest observed values were 4.67, 5.55 and 7.24 mg. The viscosity of the blood was greatly augmented, and the mean values obtained for the different groups of subjects revealed about 100 per cent increase over the corresponding values for the healthy residents; the highest observed viscosity was 32.0. The coefficient of correlation between the hematocrit level and the viscosity of the blood, calculated from all the observations made on healthy and on pathologic subjects, was $+ 0.8205 \pm 0.0227$, a coefficient similar to the one obtained by Nygaard, Wilder and Berkson [58] in investigations made at sea level.

A certain degree of equilibrium between formation and destruction of blood seems to be attained in these persons with severe anoxia and marked polycythemia; administration of phenylhydrazine to a silicotic patient who had a red cell count of 10,630,000 per cubic millimeter (the highest count observed by us at high altitudes) caused a sharp increase in the serum bilirubin followed by a definite accentuation of the reticulocytosis (chart 17). Study of the data presented in tables 22 and 23 and in charts 15 and 16 indicates that the stimulating effect of the anoxic stimulus in eliciting a polycythemic response had a well defined limit in the cases we are discussing. The greatest increases in the hemoglobin and hematocrit values were observed in men who had an arterial oxygen saturation between 70.0 and 86.0 per cent. The polycythemic hypervolemia attained its highest values in the same group of cases. With saturations of approximately 68.0 per cent or less there was a decrease in the hemoglobin and red cell volume, accompanied by a higher plasma volume (chart 18) and a greater number of reticulocytes (but in no case, regardless of the degree of anoxemia, were nucleated red cells found in the peripheral blood); the plasma bilirubin did not increase. The correlation of the arterial oxygen saturation

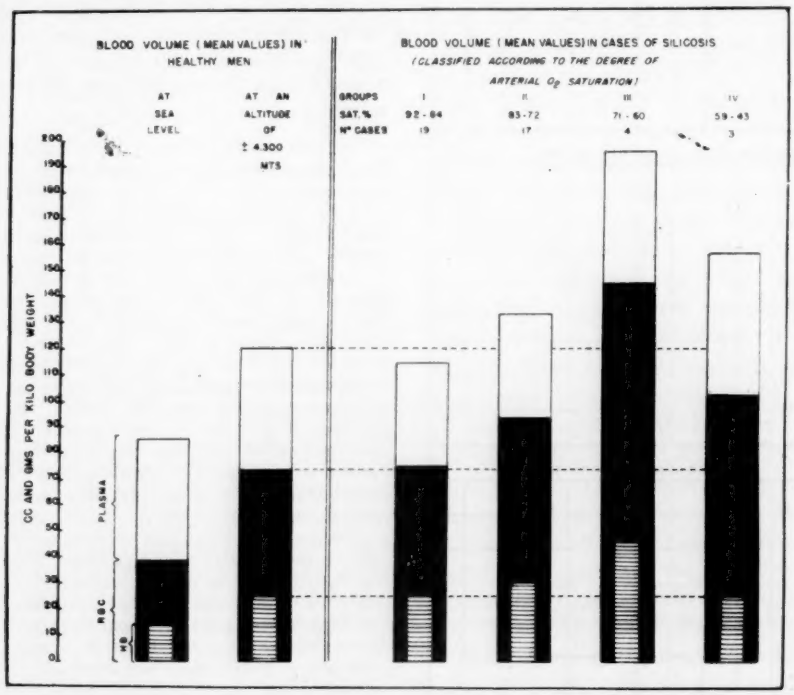
BLOOD VOLUME (MEAN VALUES) IN HEALTHY MEN
AT SEA LEVEL
AT AN ALTITUDE OF ± 4.300 MTS
BLOOD VOLUME (MEAN VALUES) IN CASES OF SILICOSIS
(CLASSIFIED ACCORDING TO THE DEGREE OF ARTERIAL O_2 SATURATION)
GROUPS I II III IV
SAT. % 92 - 84 83 - 72 71 - 60 59 - 43
Nº CASES 19 17 4 3
CC AND GMS PER KILO BODY WEIGHT
PLASMA
RBC
HB

Chart 16.—Mean blood volumes for four groups of men with silicosis studied at high altitudes, arranged according to the degree of arterial oxygen saturation. (The black zone represents red cell volume; the shaded zone, total hemoglobin; the white zone, plasma volume).

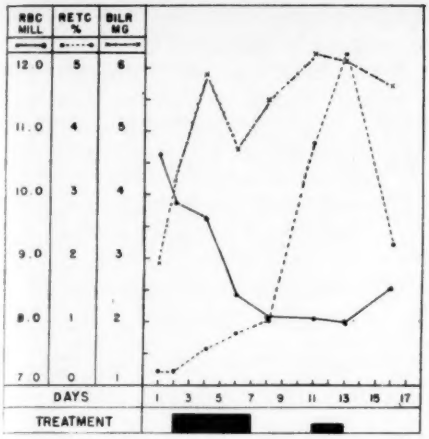

Chart 17.—Values for red blood cells, reticulocytes and bilirubin determined during the administration of phenylhydrazine to a silicotic patient with a high level of polycythemia. Observations made at high altitudes.

the mean arterial oxygen saturation was 76.2 per cent; on further ascent to an altitude of 6,140 meters (20,140 feet), which reduced the mean saturation to 65.6 per cent, a decrease in the hemoglobin and red blood cells was observed; Talbott suggested an increased rate of destruction of blood as the possible etiologic factor for this decrease.

The high level of polycythemia found in men with silicosis was not associated with a corresponding increase in the number of leukocytes per cubic millimeter (table 22). Even the subjects who had a pronounced degree of anoxemia or a high level of polycythemia failed to show significant variations in the concentration of white blood cells; the differential leukocyte count, determined for 20 of these men, revealed frequent increases in the percentage value of stab neutrophils, but in no instance were metamyelocytes observed (table 24). The lack of correlation between the number of red and of white

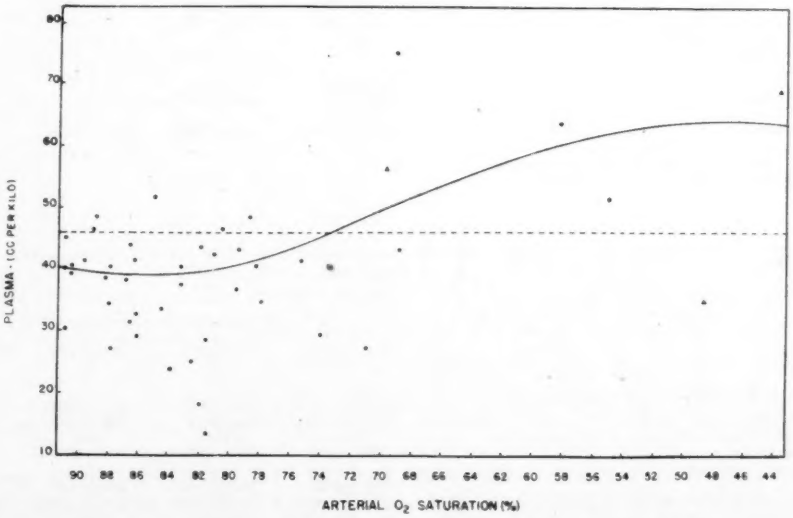

Chart 18.—Plasma volume in relation to the degree of arterial oxygen saturation in 43 patients with silicosis studied at high altitudes. The horizontal interrupted line represents the mean plasma volume for healthy native residents (determinations made with brilliant vital red dye).

with the concentration of hemoglobin in the circulating erythrocytes revealed that an increasing degree of anoxemia was associated with a gradual diminution in the amount of hemoglobin held by the red cells (chart 19); in this connection it was also significant to find a decreased mean corpuscular volume in the men who had an unusually high arterial insaturation (table 22). The observations made by Talbott [56] on the members of the High Altitude Expedition to the Chilean Andes have a related significance; the highest level of polycythemia was found at an elevation of 5,340 meters (17,500 feet), where blood cells in the peripheral blood and the absence of leukocytosis as a response to a condition of chronic anoxemia may be appreciated in chart 20.

Chemical study of the gastric contents in hematologic disorders has attained a considerable interest in recent years. Most of this relates to the causative mechanism of various anemic processes, but it has been suggested [71] that variations in the gastric acid content induce

71. Lombardi, E., and Cino, J. A.: Riforma med. **52**:1343, 1936.

TABLE 22.—*Observations on Blood of Patients with Silicosis Grouped According to the Degree of Anoxemia (Study Made at an Altitude of 3,730 Meters; 12,240 Feet)*

	Arterial O_2 Saturation			
	84% or More	72.0-83.9 %	60.0-71.9 %	Less Than 60.0 %
Number of cases	40	33	5*	4†
Red blood cells (millions per cu. mm.)				
Mean ± P. E.	6.65 ± 0.10	7.72 ± 0.09	7.90 ± 0.49	7.98 ± 0.53
Standard deviation ± P. E.	0.90 ± 0.07	0.80 ± 0.06	1.47 ± 0.35	1.35 ± 0.37
Coefficient of variation (%)	13.5	10.3	18.4	16.9
Extreme variations	4.58 - 8.36	5.64 - 9.22	6.46 - 10.63	5.83 - 9.66
Hematocrit reading (red cells, per cent)				
Mean ± P. E.	63.7 ± 0.65	71.6 ± 0.82	74.7 ± 2.12	67.9 ± 2.34
Standard deviation ± P. E.	6.1 ± 0.46	7.0 ± 0.58	6.3 ± 1.50	6.0 ± 1.65
Coefficient of variation (%)	9.5	9.8	8.4	8.8
Extreme variations	51.8 - 80.1	58.7 - 81.2	66.2 - 82.3	58.5 - 74.1
Hemoglobin (Gm. per 100 cc.)				
Mean ± P. E.	21.52 ± 0.19	23.77 ± 0.24	24.26 ± 0.85	20.58 ± 0.72
Standard deviation ± P. E.	1.87 ± 0.14	2.04 ± 0.17	2.53 ± 0.60	1.86 ± 0.51
Coefficient of variation (%)	8.6	8.5	10.4	9.0
Extreme variations	18.03 - 26.64	19.23 - 27.47	21.77 - 28.68	17.66 - 22.72
Corpuscular mean volume (cu. microns)				
Mean ± P. E.	96.7 ± 1.19	92.8 ± 1.00	95.4 ± 4.15	88.2 ± 8.03
Standard deviation ± P. E.	11.2 ± 0.84	8.2 ± 0.68	12.3 ± 2.93	20.6 ± 5.60
Coefficient of variation (%)	11.5	8.8	12.9	23.3
Extreme variations	80.0 - 120.9	77.1 - 106.2	76.4 - 109.9	72.4 - 128.7
Corpuscular mean hemoglobin (micromicrograms)				
Mean ± P. E.	32.7 ± 0.33	31.1 ± 0.34	31.1 ± 1.69	26.9 ± 2.72
Standard deviation ± P. E.	3.1 ± 0.23	2.9 ± 0.24	5.0 ± 1.19	7.0 ± 1.92
Coefficient of variation (%)	9.4	9.3	16.0	26.0
Extreme variations	27.5 - 41.3	26.1 - 38.6	23.5 - 35.7	21.6 - 38.9
Corpuscular mean hemoglobin concentration (%)				
Mean ± P. E.	33.9 ± 0.09	33.3 ± 0.13	32.4 ± 0.50	30.3 ± 0.54
Standard deviation ± P. E.	0.9 ± 0.07	1.1 ± 0.09	1.5 ± 0.36	1.4 ± 0.38
Coefficient of variation (%)	2.6	3.3	4.6	4.6
Extreme variations	31.4 - 35.4	31.2 - 36.7	30.0 - 34.9	28.2 - 31.5
Reticulocytes (per cent)				
Mean ± P. E.	0.6 ± 0.07	0.7 ± 0.06	0.6 ± 0.20	1.9 ± 0.27
Standard deviation ± P. E.	0.5 ± 0.05	0.5 ± 0.04	0.6 ± 0.14	0.7 ± 0.19
Coefficient of variation (%)	83.3	71.4	100.0	36.8
Extreme variations	0 - 1.4	0 - 2.2	0.2 - 2.0	0.6 - 2.4
Leukocytes (per cu. mm.)				
Mean ± P. E.	6,600 ± 220	6,770 ± 173	6,350 ± 779	7,330 ± 604
Standard deviation ± P. E.	2,060 ± 155	1,480 ± 122	2,310 ± 552	1,550 ± 426
Coefficient of variation (%)	31.2	21.7	36.3	21.1
Extreme variations	4,640 - 18,280	4,360 - 17,200	3,680 - 10,600	5,360 - 8,99

* Includes 1 patient with circulatory insufficiency.
† Includes 2 patients with circulatory insufficiency.

TABLE 23.—*Observations on Blood of Patients with Silicosis Grouped According to the Degree of Anoxemia (Study Made at an Altitude of 3,730 Meters; 12,240 Feet)*

	Arterial O_2 Saturation			
	84% or More	72.0-83.9 %	60.0-71.9 %	Less Than 60.0 %
Number of cases	19	17	4*	3†
Blood volume (cc. per Kg.)				
Mean ± P. E.	114.6 ± 2.72	133.5 ± 4.27	191.3 ± 21.6	156.6 ± 5.06
Standard deviation ± P. E.	17.6 ± 1.92	26.1 ± 3.01	55.6 ± 14.8	10.6 ± 3.57
Coefficient of variation (%)	15.3	19.5	29.1	6.7
Extreme variations	78.3 - 153.5	92.9 - 197.9	131.0 - 280.4	143.1 - 169.0
Plasma volume (cc. per Kg.)				
Mean ± P. E.	39.3 ± 1.05	37.3 ± 0.70	45.0 ± 4.29	52.3 ± 6.59
Standard deviation ± P. E.	6.8 ± 0.74	4.3 ± 0.49	11.0 ± 3.03	13.6 ± 4.59
Coefficient of variation (%)	17.3	11.5	24.4	26.0
Extreme variations	27.3 - 52.3	19.1 - 49.1	27.9 - 57.3	35.9 - 65.3
Red cell volume (cc. per Kg.)				
Mean ± P. E.	75.0 ± 2.52	94.1 ± 3.89	145.3 ± 19.9	103.2 ± 1.72
Standard deviation ± P. E.	16.3 ± 1.78	23.8 ± 2.75	51.1 ± 14.0	3.6 ± 1.21
Coefficient of variation (%)	21.7	25.3	35.1	3.5
Extreme variations	43.3 - 109.8	64.3 - 156.1	86.8 - 227.5	98.1 - 106.1
Total Hemoglobin (Gm. per Kg.)				
Mean ± P. E.	25.1 ± 0.74	30.9 ± 1.03	46.7 ± 5.75	31.0 ± 0.42
Standard deviation ± P. E.	4.8 ± 0.53	6.4 ± 0.73	15.0 ± 4.13	0.9 ± 0.30
Coefficient of variation (%)	19.1	20.7	32.1	2.9
Extreme variations	14.7 - 36.7	22.1 - 51.2	28.6 - 70.1	29.8 - 33.3
Bilirubin (mg. per 100 cc.)				
Mean ± P. E.	2.30 ± 0.15	3.00 ± 0.28	2.30 ± 0.19	1.64 ± 0.30
Standard deviation ± P. E.	1.00 ± 0.11	1.71 ± 0.19	0.49 ± 0.13	0.64 ± 0.21
Coefficient of variation (%)	43.5	57.0	21.3	39.0
Extreme variations	1.00 - 4.13	0.91 - 7.24	1.76 - 2.88	0.74 - 2.21
Blood viscosity				
Mean ± P. E.	15.8 ± 0.83	20.7 ± 0.95	19.6 ± 2.56	16.6 ± 2.88
Standard deviation ± P. E.	3.4 ± 0.59	5.8 ± 0.67	7.6 ± 1.81	7.4 ± 2.03
Coefficient of variation (%)	34.2	28.0	38.8	44.6
Extreme variations	9.0 - 30.0	11.4 - 32.0	7.2 - 28.8	10.2 - 28.6

* Includes 1 patient with circulatory insufficiency.
† Includes 2 patients with circulatory insufficiency.

parallel changes in the erythropoiesis. We have investigated the gastric acidity of 9 patients with silicosis, all with pronounced polycythemia, employing a 7 per cent solution of alcohol and 1 cc. of histamine phosphate (Parke, Davis & Company) as stimulants for the gastric mucosa (table 25). Free hydrochloric acid and pepsin were absent in all residual samples; in 5 cases free hydrochloric acid failed to appear after the ingestion of alcohol, and this condition persisted in 4 cases after injection of histamine. Previous observations, not published, made by one of us (A. H.) are related to these findings; of 37 native Indian residents of Morococha (at 4,540 meters; 14,900 feet) who were examined on account of various gastric symptoms (discomfort, indigestion, etc.) an absence of free hydrochloric acid was verified in 21, equivalent to 56.7 per cent of the total. These results suggest that achlorhydria, and possibly achylia gastrica, is not an infrequent finding in residents of high altitudes, and that the polycythemia induced by the anoxic stimulus may coexist with this chemical change in the gastric contents. A similar observation has been made at sea level in a case of polycythemia vera.[72]

Observations in Cases of Chronic Mountain Sickness.—Monge,[73] in 1928, reported that loss of tolerance to a low barometric pressure environment after a constant or prolonged residence is frequently associated with an abnormal accentuation of the polycythemia which in some degree is found in most dwellers at high altitudes. The different aspects of this hematologic disorder, to which the names chronic mountain sickness, high altitude erythremia and Monge's disease have been given, have been recently reviewed by Monge.[74] Table 26 shows the hematologic characteristics and the degree of arterial oxygen saturation of 8 patients with this condition, whose cases have been described in greater detail in a recent paper.[75] When compared with the healthy residents of the altitudes at which they lived, all these patients showed a considerable increase in the hemoglobin and red cell volume, associated in most cases with a decrease in the plasma volume. The bilirubin was elevated in all the cases in which it was determined; in 2 cases the reticulocyte count reached high values. The leukocyte count was found to be within normal limits. These observations confirmed previous studies.[76] In all but 1 of the cases the arterial oxygen saturation was below the limits of variation found in healthy residents. Three of the 4 patients who were followed after descent to sea level had a decrease of the cell volume associated with a less proportional increase in the plasma volume.

In 3 cases of chronic mountain sickness in which the patients were studied after descent to sea level, Urteaga and Boisset[77] observed an

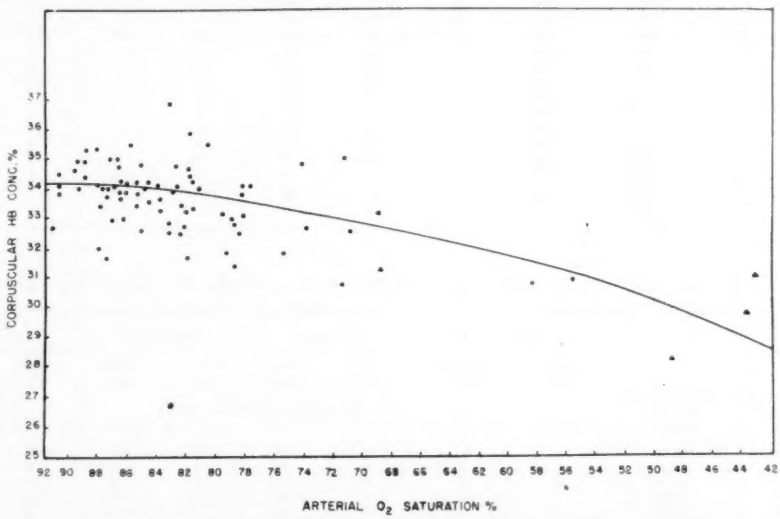

Chart 19.—Relationship between the mean corpuscular hemoglobin concentration and the degree of arterial oxygen saturation in men with silicosis studied at high altitudes.

72. Michaelidis, F.: Wien. klin. Wchnschr. **45**:1250, 1932.

73. Monge, C., and others: Crón. méd., Lima **45**: 238, 282 and 345, 1928; **46**:13, 58, 80 and 129, 1929.

74. Monge, C.: Physiol. Rev. **23**:166, 1943.

75. Hurtado, A.: Chronic Mountain Sickness, J. A. M. A. **120**:1278 (Dec. 19) 1942.

76. Hurtado, A.: Aspectos fisiológicos y patológicos de la vida en la altura, Lima, Editorial Rimac, 1937.

77. Urteaga, O., and Boisset, G.: An. Fac. de med., Lima **25**:67, 1942.

increase in the hemoglobin and hematocrit values and in the total cell volume and a decrease in the plasma volume. The bilirubin content was elevated, and the test for excretion of this pigment revealed an abnormal retention. However, roentgen examination disclosed the presence of

conditions, which may show analogous hematologic alterations on account of the increased anoxic stimulus present in both but which are two entirely separate entities, already has been emphasized,[75] and this is especially important in studies carried out in mining zones located at high altitudes.

TABLE 24.—*Leukocytes and Leukocytic Differential Counts of Patients with Silicosis with a High Degree of Anoxemia or Polycythemia (Observations Made at an Altitude of 3,730 Meters; 12,240 Feet)*

Case No.	Arterial O_2 Saturation, %	Hemoglobin, Gm. per 100 Cc.	Hematocrit Reading, R.B.C., %	Leukocytes, per Cu. Mm.	Neutrophils, Stab, %	Neutrophils, Segmented, %	Neutrophils, Total, %	Eosinophils, %	Basophils, %	Monocytes, %	Lymphocytes, %
1	43.3	22.72	72.1	8,720	28	60	88	0	0	4	8
2	48.9	20.82	74.1	6,280	7	76	83	0	1	5	11
3	43.9	17.66	58.5	5,360	20	66	86	1	0	4	9
4	71.2	28.68	82.3	3,680	8	65	73	1	3	10	13
5	68.8	21.77	69.6	5,200	20	68	88	0	0	4	8
6	71.4	25.00	81.2	6,400	1	65	66	0	0	9	25
7	75.3	23.07	72.6	5,280	9	71	80	3	0	2	15
8	83.2	24.04	65.4	5,960	14	39	53	3	1	9	34
9	86.1	23.10	68.2	6,000	16	52	68	4	0	3	25
10	79.5	25.30	76.5	6,760	4	71	75	1	0	7	17
11	78.1	24.78	73.0	5,580	14	64	78	0	0	4	18
12	82.1	26.43	81.1	7,600	8	65	73	1	1	9	16
13	82.4	24.93	76.9	6,400	10	63	73	1	0	2	24
14	90.8	23.92	68.6	7,880	4	71	75	1	1	1	22
15	78.3	24.22	75.0	7,800	2	65	67	2	3	5	23
16	83.2	25.85	78.8	4,720	11	47	58	2	0	6	34
17	78.8	25.38	77.2	11,200	7	49	56	7	2	3	32
18	78.2	26.89	81.2	5,800	4	46	50	2	0	3	45
19	86.5	24.45	71.6	7,600	8	48	56	5	0	8	31
20	87.8	26.64	80.1	5,600	5	46	51	2	0	3	44

TABLE 25.—*Gastric Acidity of Patients with Silicosis Studied at an Altitude of 3,730 Meters (12,240 Feet)*

Case No.	Arterial O_2 Saturation, %	Hemoglobin, Gm. per 100 Cc.	Sample 1		Sample 2		Sample 3		Sample 4	
			Free HCl	Total Acid	Free HCl	Total Acid	Free HCl	Total Acid	Free HCl	Total Acid
			Cc. of N/10 NaOH per 100 Cc.							
1	85.4	23.79	0	10	13	26	15	32	22	36
2	83.1	24.05	0	5	0	10	4	4	3	16
3	79.2	22.87	0	9	0	5	0	7	0	8
4	82.9	27.47	0	12	20	32	32	40	34	42
5	70.8	24.01	0	3	0	4	0	4	0	4
6	77.7	26.28	0	8	0	10	0	10	0	8
7	87.4	20.15	0	6	0	8	10	18	16	24
8	78.2	23.98	0	4	0	6	0	8	0	10
9	86.6	22.55	18	30	16	24	24	34	38	46

Sample 1, residual.
Sample 2, 20 minutes after 50 cc. of 7 per cent alcohol.
Sample 3, 40 minutes after 50 cc. of 7 per cent alcohol.
Sample 3, 15 minutes after injection of histamine.

TABLE 26.—*Observations on Blood of Persons with Chronic Mountain Sickness*

Case No.	Place of Study, Altitude, Meters	Arterial O_2 Saturation, %	Red Blood Cells, Millions per Cu. Mm.	Hematocrit, R.B.C., %	Hemoglobin, Gm. per 100 Cc.	Reticulocytes, per Cent	Bilirubin, Mg. per 100 Cc.	Leukocytes, per Cu. Mm.	Blood Volume, Cc. per Kg.	Plasma Volume, Cc. per Kg.	Red Cell Volume, Cc. per Kg.	Total Hemoglobin, Gm. per Kg.
1	3,730	77.4	9.35	83.0	27.17	4.4	8.33	3,800	202.9	33.4	168.3	55.1
2	3,730	82.9	7.37	74.6	25.57	1.2	2.24	6,400	157.1	37.1	111.1	38.1
3	3,730	75.9	8.63	73.0	24.01	0.6	5.55	6,210	149.6	39.6	109.2	35.9
4	3,730	79.4	8.50	81.7	24.60	5.6	2.67	7,200	211.9	27.7	173.1	52.1
5	4,540	76.1	7.70	73.7	22.89	0.8	3.52	6,800	167.2	43.1	123.3	38.4
6	4,540	77.3	7.61	75.7	25.73	0.8	....	5,800	196.6	46.8	148.8	50.6
7	Sea level	95.9	8.00	79.2	26.13	0.3	4.50	6,100	186.2	37.8	147.3	48.6
8	Sea level	91.2	8.97	79.2	23.50	0	....	6,400	197.8	40.2	156.7	46.5

silicosis in 2 of the 3 subjects; so the reported findings in these cases may be related to this illness rather than to chronic mountain sickness. The importance of differentiating between these two

ROLE OF ANOXEMIA IN POLYCYTHEMIA OBSERVED AT SEA LEVEL

Polycythemia frequently accompanies chronic pulmonary diseases in which there is some degree

of arterial oxygen unsaturation. Such hematic alteration has been observed in subjects with fibrous changes in the lungs due to inhalation of dust,[78] with chronic bronchitis, asthma or emphysema,[79] with gas poisoning [80] and with primary pulmonary arteriosclerosis of unknown origin.[81] The polycythemia which is found in association with Ayerza's disease, characterized by widespread fibrosclerotic pulmonary changes and right-sided heart failure,[82] is well known. Our observations at high altitudes give further support to the generally accepted view that the polycythemia associated with such conditions represents a response to the anoxic stimulus. It is difficult, however, to establish a precise comparison between the polycythemia that develops at high altitudes and that observed with the diseases just mentioned, on account of the lack of information regarding the occurrence and the degree of anoxemia in most of the reported cases. From the data reported by Berconsky,[82a] Capdehourat [82b] and Hurtado, Kaltreider and McCann [83] we have constructed the diagram of chart 21, which shows the relationship between the degree of arterial oxygen unsaturation and the amount of circulating hemoglobin in cases of pulmonary fibrosis, emphysema and Ayerza's disease. It appears evident that most of the subjects with an arterial oxygen saturation of less than 90 per cent have some increase in hemoglobin, but this hematic response tends to be less than in residents of high altitudes with a corresponding degree of unsaturation and is frequently absent, especially in persons with pulmonary emphysema. The patients with Ayerza's disease have the highest increases in hemoglobin; of 18 studied by the investigators mentioned, 8, or 44.4 per cent, had values over 20 Gm. per hundred cubic centimeters. The frequent lack of erythropoietic response to the anoxemia which is usually associated with pulmonary emphysema has been previously pointed out.[84]

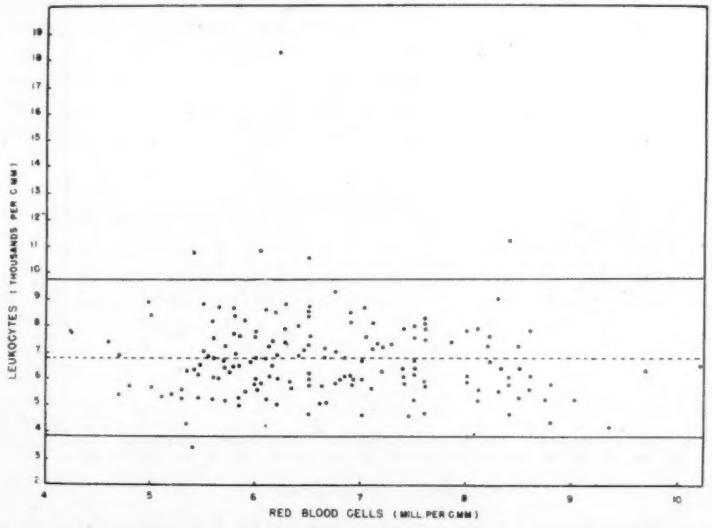

Chart 20.—Leukocyte count in relation to the erythrocyte count in healthy and pathologic subjects studied at high altitudes. The zone between the horizontal lines represents the limits of variation observed in healthy men at sea level.

The moderate degree of anoxemia, which has been found to be of common occurrence after the age of 60 years [85] not only fails to elicit a polycythemic response, but, according to recent observations,[86] is accompanied by low red cell counts and hemoglobin values.

78. Barker, N. W.: Polycythemia Vera and Chronic Pulmonary Disease, Arch. Int. Med. **47**:94 (Jan.) 1931. Kaltreider, Hurtado and Brooks.[36g]

79. Waring, J. J., and Yegge, W. B.: Ann. Int. Med. **7**:190, 1933.

80. Barcroft, J.; Hunt, G. H., and Dufton, D.: Quart. J. Med. **13**:179, 1920.

81. Darley, W., and Doan, C. A.: Am. J. M. Sc. **191**:633, 1936.

82. (*a*) Berconsky, I.: Semana méd. **1**:1569, 1933. (*b*) Capdehourat, E. L.: La cianosis de los cardiacos negros de Ayerza, Buenos Aires, Aniceto Lopez, 1934. (*c*) Jiménez Díaz, C.; Centenera, D., and Alemany, M.: Arch. cardiol. y hemat. **16**:306, 1935.

83. Hurtado, A.; Kaltreider, N. L., and McCann, W. S.: J. Clin. Investigation **14**:94, 1935.

84. Lemon, W. S.: Ann. Int. Med. **3**:430, 1929. Kaltreider, Hurtado and Brooks.[36g]

85. Dill, D. B.; Graybiel, A.; Hurtado, A., and Taquini, A. C.: Ztschr. f. Altersforsch. **2**:20, 1940.

86. Newman, B., and Gitlow, S.: Am. J. M. Sc. **205**:677, 1943.

There is rather scanty information in the literature regarding the blood volume and the morphologic characteristics of the erythrocytes in polycythemia secondary to pulmonary disease. It has been pointed out [36d] that the increase in blood volume is moderate; however, values as high as 136 and 168 cc. of blood per kilogram of body weight have been reported.[36g] The investigation of the blood volume in cases of Ayerza's disease, in which the hemoglobin reaches the highest values observed with secondary polycythemia at sea level, will be of considerable interest. Kaltreider, Hurtado and Brooks [36g] found an unchanged corpuscular volume in cases of pulmonary fibrosis associated with some degree of polycythemia, but the size of the red blood cells had an inverse correlation with their number per cubic millimeter; with counts over 6,000,000 and 6,500,000 the mean corpuscular volume was found to be 81.4 and 74.3 cubic microns, respectively, figures which are distinctly lower than those found by us for residents of high altitudes with similar levels of polycythemia. An increase in the total mass of red cells with a reduction in the plasma volume, a finding similar to ours in the silicotic patients studied at high altitudes, has been recently reported by Hallock [86k] in cases of congenital heart disease of the cyanotic type. Less clear appears to be the mechanism underlying the moderate polycythemia which has been frequently observed in cases of heart failure with a normal arterial oxygen saturation [87]; an anoxia of the circulatory type is probably the main factor responsible for the bone marrow stimulation in these cases. Gibson and Evans [88] have found that the degree of increase in the blood volume in cases of circulatory insufficiency parallels the elevation of the venous blood pressure and the slowing of the circulation time. It is significant that increase in plasma volume such as was noted in their subjects at sea level has been observed by us at high altitudes in persons with silicosis associated with heart failure. A certain degree of polycythemia has been frequently observed in newborn infants,[89] and it is probably related, as has been suggested,[90] to the low oxygen saturation which has been demonstrated to exist during fetal life and at birth.[91] The red blood cells of

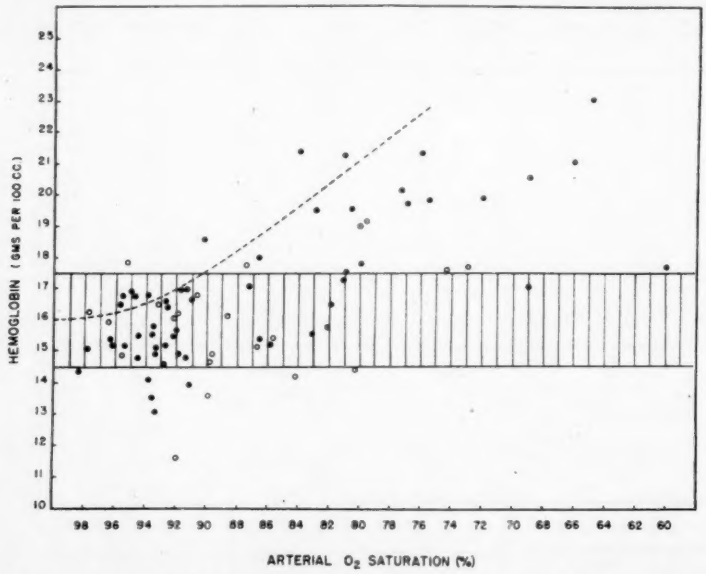

Chart 21.—Relation between the hemoglobin content (grams per hundred cubic centimeters) and the degree of arterial oxygen saturation (per cent) in persons with fibrosis (solid dots), emphysema (open circles) and Ayerza's disease (dotted circles) studied at sea level. Data obtained from the literature [82] and from previous investigations.[83] The shaded area shows variations in the blood hemoglobin of healthy men living at sea level; the interrupted curve represents the approximate mean hemoglobin values which are found at various degrees of arterial oxygen saturation in healthy residents at high altitudes.

87. Harrison, T. R.: Failure of the Circulation, Baltimore, Williams & Wilkins Company, 1936. Altschule, M. D.: Medicine **17**:75, 1938.

88. Gibson, J. G., and Evans, W. A.: J. Clin. Investigation **16**:851, 1937.

89. Harrop, G., and Wintrobe, M. M.: Polycythemia, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 4.

90. Goldbloom, A., and Gottlieb, R.: J. Clin. Investigation **8**:375, 1930.

91. Barcroft, J.; Kramer, K., and Millikan, T. A.: J. Physiol. **90**:28P, 1937. Barcroft, J., and Mason, M. F.: ibid. **93**:32P, 1938.

newborn infants are larger and have a greater surface area than those of adults but the corpuscular hemoglobin concentration is identical with that of adults [92]—morphologic characteristics which are similar to those observed by us in subjects constantly exposed to a low barometric pressure. The average corpuscular volume of 95 cubic microns found by Chuinard, Osgood and Ellis [92b] in a study of 195 newborn infants has a close similarity to the mean values of 95.2 ± 0.58 and 97.5 ± 0.75 cubic microns obtained for the Indian residents of Oroya (at 3,740 meters [12,240 feet] of altitude) and Morococha (at 4,540 meters [14,900 feet]) respectively. Wintrobe and Shumacker [93] have observed a high degree of macrocytosis associated with a low red cell count in the blood of fetuses. The high bilirubin content of the plasma in the newborn has been explained on the basis of a high degree of destruction of blood [94] and of a decreased ability of the liver cells to excrete the pigment,[95] factors which must be also taken into consideration to explain the hyperbilirubinemia associated with the chronic anoxemia of high altitudes.

Several investigations recently reviewed by Killick [50] have demonstrated the occurrence of polycythemia in human beings and animals with carbon monoxide anoxemia. The observations of Sayers, Yant, Levy and Fulton,[96] who found increases in the red blood cells and hemoglobin of men who had repeated daily exposures to carbon monoxide (contained in automobile exhaust gas), have a significance related to the polycythemia we have observed in men subject to the influence of intermittent periods of anoxemia at high altitudes. Some polycythemias produced experimentally in animals have been attributed to anoxic factors with a resulting stimulation of the erythropoietic activity. The polycythemia that follows the administration of cobalt, which is absolute in type,[97] seems to be related to an interference with cellular respiration.[98] The increases in the red cell count and hemoglobin produced by pressor drugs (epinephrine, ephedrine and amphetamine) have been explained on the basis of an abnormally low supply of oxygen to the bone marrow caused by a decrease in blood flow.[99]

The most important polycythemic process at sea level is undoubtedly polycythemia vera, or Vasquez' disease, characterized by a considerable in-

TABLE 27.—*Level of Polycythemia, Reticulocytosis and Bilirubinemia in Relation to the Degree and Constancy of the Anoxemia (Summary of the Observations Made on Different Groups of Male Subjects)*

Groups	No. of Subjects	Altitude		Arterial O_2 Saturation, %	Hemoglobin, Gm. per 100 Cc.	Reticulocytes, per Cent	Serum Bilirubin			Ratio, D/T × 100
		Meters	Feet				Mg. per 100 Cc.			
							Total	Direct	Indirect	
				Mean ± P. E.						
I. At sea level	175	0	0	96.1 ± 0.12	16.00 ± 0.04	0.5 ± 0.02	0.72 ± 0.02	0.37 ± 0.01	0.35 ± 0.01	51.4
II. Temporary anoxemia										
(*a*) 2 hours' exposure	15	2,390	7,920	91.0 ± 0.57	16.22 ± 0.16	0.5 ± 0.05	0.76 ± 0.04	0.45 ± 0.03	0.31 ± 0.02	59.2
(*b*) 2 hours' exposure	16	3,140	10,300	89.6 ± 0.74	16.31 ± 0.15	0.5 ± 0.08	0.67 ± 0.04	0.36 ± 0.02	0.31 ± 0.03	53.7
(*c*) 2 hours' exposure	18	4,165	13,660	80.2 ± 0.77	16.20 ± 0.15	0.4 ± 0.05	0.74 ± 0.04	0.36 ± 0.02	0.38 ± 0.03	48.6
(*d*) 2 hours' exposure	18	4,835	15,870	75.3 ± 0.93	16.53 ± 0.18	0.4 ± 0.04	0.79 ± 0.07	0.39 ± 0.03	0.40 ± 0.04	49.4
III. Intermittent anoxemia										
(*a*) Infrequent exposure *	60	.....	.....	.....	16.53 ± 0.09	0.7 ± 0.05	1.00 ± 0.06	0.43 ± 0.02	0.56 ± 0.05	43.0
(*b*) Daily exposure †	13	.....	.....	.....	18.07 ± 0.28	0.8 ± 0.11	1.16 ± 0.15	0.40 ± 0.03	0.76 ± 0.13	34.5
IV. Chronic anoxemia										
(*a*) Native residents	40	3,730	12,240	87.6 ± 0.27	18.82 ± 0.15	0.8 ± 0.06	1.47 ± 0.09	.....	.....	....
(*b*) Native residents	32	4,540	14,900	81.4 ± 0.45	20.76 ± 0.20	1.5 ± 0.07	1.56 ± 0.19	0.46 ± 0.03	1.10 ± 0.17	29.5

* Flight personnel (see text).
† Railroad personnel (see text).

92. (*a*) Mugrage, E. R., and Andresen, M. I.: Values for Red Blood Cells of Average Infants and Children, Am. J. Dis. Child. **51**:775 (April) 1936. (*b*) Chuinard, E. G.; Osgood, E. E., and Ellis, D. M.: Hematologic Standards for Healthy Newborn Infants: Erythrocyte Count, Hemoglobin Content, Cell Volume, Color Index, Volume Index and Saturation Index, Am. J. Dis. Child. **62**:1188 (Dec.) 1941.

93. Wintrobe, M. M., and Shumacker, H. B.: J. Clin. Investigation **14**:837, 1935.

94. Gordon, M. B., and Kemelhor, M. C.: J. Pediat. **2**:685, 1933. Rolleston, H., and McKee, J. W.: Diseases of the Liver, London, The Macmillan Company, 1929. Ehrenfest, H.: Causation of Intracranial Hemorrhages in New-Born, Am. J. Dis. Child. **26**:503 (Dec.) 1923.

95. Snelling, C. J.: J. Pediat. **2**:399, 1933. Waugh, T. R.; Merchant, F. T., and Maughan, G. B.: Am. J. M. Sc. **199**:9, 1940.

96. Sayers, R. R.; Yant, W. P.; Levy, E., and Fulton, W. B.: Effect of Repeated Daily Exposure of Several Hours to Small Amounts of Automobile Exhaust Gas, Public Health Bulletin 186, United States Treasury Department, Public Health Service, 1929

97. Davis, J. E.: Proc. Soc. Exper. Biol. & Med. **45**:671, 1940.

98. Davis, J. E.: J. Pharmacol. & Exper. Therap. **70**:408, 1940. Orten, J. M.: Detroit M. News (Educ. Issue) **32**:42, 1941.

99. Davis, J. E.: Am. J. Physiol. **133**:258, 1941; **134**:219, 1941.

crease in the circulating blood volume. The cause of this disease is unknown, but among the various theories which have been advanced those related to the possible existence of an anoxic stimulating factor have a prominent place. All investigators [100] have reported normal values for the oxygen saturation of the arterial blood, but Harrop and Heath [101] found a reduction following exercise, which they attributed to a decreased permeability of the alveolar membrane for the passage of oxygen; to the anoxemia thus produced a causative significance was attributed. Reznikoff, Foot and Bethea [102] have found in cases of this disease a marked thickening of the capillaries and fibrosis of the arterioles in the bone marrow, changes which have led them to propose the theory that the erythremia may be due to a local oxygen deficiency in the erythropoietic organs, with overcompensation of erythrogenesis. Hallock,[103] from observations related to the production of lactic acid during exercise, suggested that tissue anoxia may be an important causative factor of polycythemia vera.

The increase in the circulating blood volume, due to a greater mass of red cells with a practically unchanged or decreased plasma volume, is a characteristic common to the polycythemia observed at high altitudes and polycythemia vera. However, a comparative study of the results obtained by several investigators [104] in the study of a total of 91 subjects (males) with the latter disease and our findings in 97 healthy residents and persons with silicosis at high altitudes reveals that the increase in blood volume is more pronounced and has a greater range of variation in the patients with polycythemia vera (chart 22). The mean value for all reviewed cases of this disease was 154.1 ± 2.73 cc. of blood per kilogram of body weight, with a standard deviation of 38.5 cc., while in the 97 of polycythemia at high altitudes the mean volume was 120.6 ± 1.72 with a standard deviation of 25.1 cc.

There are certain other comparative aspects which have interest from the point of view of the etiologic mechanisms responsible for the polycythemic processes in these two groups of cases. A statistical analysis of the data published by Rowntree and Brown, Brown and Giffin [105] and Brown and Roth,[106] which refer to observations made on a total of 62 male patients with polycythemia vera, gives mean values of 22.56 ± 0.30 Gm. of hemoglobin per hundred cubic centimeters and 63.7 ± 0.61 red cells per cent for the hematocrit value. According to our observations, these mean values would correspond, approximately, to those found in men living at an altitude of 5,300 meters (17,400 feet) with an arterial oxygen saturation of about 76.0 per cent.

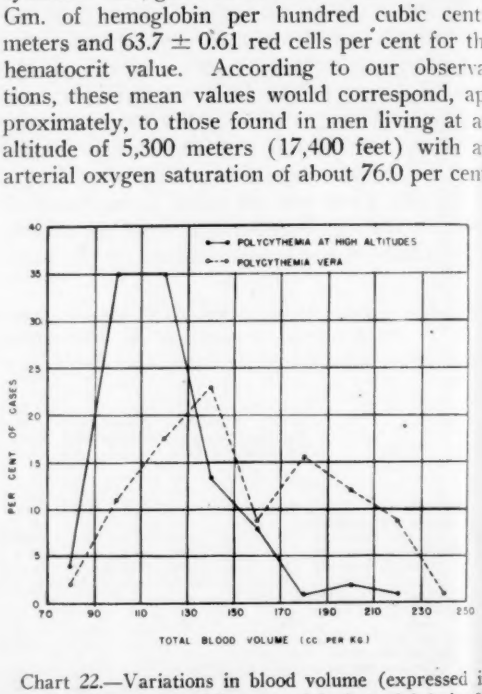

Chart 22.—Variations in blood volume (expressed in cubic centimeters per kilogram of body weight) in 97 healthy and pathologic subjects studied at high altitudes (with arterial oxygen saturations ranging from 43.3 to 90.2 per cent) and in 91 persons with polycythemia vera observed at sea level. The data corresponding to the latter disease have been collected from the literature.[105]

The size of the red blood cells has been found to be normal or moderately decreased in most cases of polycythemia vera,[89] and microcytes are often observed [107]; in the series of 62 cases to which we have just referred, the mean corpuscular volume and hemoglobin concentration were found to be 92.2 ± 0.94 cubic microns and 36.4 ± 0.24 per cent, respectively. These figures are not unlike those found by us in polycythemia of high altitudes, but it appears that in the latter process there is a deviation toward

100. Altschule, M. D.; Volk, M. C., and Henstell, H.: Am. J. M. Sc. **200**:478, 1940.

101. Harrop, G. A., and Heath, E. H.: J. Clin. Investigation **4**:53, 1927.

102. Reznikoff, P.; Foot, N. C., and Bethea, J. M.: Am. J. M. Sc. **189**:753, 1935.

103. Hallock, P.: Proc. Soc. Exper. Biol. & Med. **38**:587, 1938.

104. Haden, R. L.: Am. J. M. Sc. **196**:493, 1938. Sohval, A. R.: Hepatic Complications in Polycythaemia Vera, with Particular Reference to Thrombosis of Hepatic and Portal Veins and Hepatic Cirrhosis, Arch. Int. Med. **62**:925 (Dec.) 1938. Gibson, J. G.; Harris, A. W., and Swigert, V. W.: J. Clin. Investigation **18**:621, 1939. Meyer, O. O., and Thewlis, E. W.: J. Lab. & Clin. Med. **26**:1137, 1941. Rountree and Brown.[36d] Goldbloom and Libin.[36h] Hallock.[36k] Altschule and others.[100]

105. Brown, G. E., and Giffin, H. Z.: Treatment of Polycythemia Vera (Erythremia) with Phenylhydrazine, Arch. Int. Med. **38**:321 (Sept.) 1926.

106. Brown, G. E., and Roth, G. M.: J. Clin. Investigation **6**:159, 1928.

107. Schalm, L.: Folia haemat. **63**:34, 1939. Wintrobe.[20]

a slight macrocytosis which is not observed in polycythemia vera. Nucleated red cells in the peripheral blood are not infrequently noted in this disease,[108] a finding not verified by us in the case of any subject studied at high altitudes, regardless of the degree of anoxemia and the level of polycythemia. The increase in the serum bilirubin seems to be more intense and frequent in polycythemia of high altitudes, as may be judged from the comparison of our data with those contained in recent monographs on polycythemia vera.[89]

Another distinction, which is probably the most important and of fundamental significance, relates to the great frequency of leukocytosis and leukemia, with appearance of immature white cells in the peripheral blood, in cases of polycythemia vera; this characteristic has been emphasized by all writers. With the exception of a moderate and temporary leukocytosis which is observed at times during the early period of exposure to a low barometric pressure and probably is due to factors of mobilization and release, there is no disturbance of the leukoblastic activity in the polycythemia of high altitudes, no matter how intense and constant are the anoxic stimulus and the degree of erythropoietic response. Finally, an important clinical distinction is the absence of splenomegaly in healthy and in diseased polycythemic subjects living at high altitudes, in contrast with its frequency in persons with polycythemia vera; according to a recent study [109] the increased size of the spleen was clinically verified in about 66 per cent of 163 cases of the latter disease. A somewhat closer similarity with the polycythemia of healthy persons living at high altitudes is found in the so-called familial polycythemia, in which the increase in the total cell volume is associated with an unchanged or moderately decreased plasma volume, normal morphologic characteristics of the red cells and absence of leukocytosis and changes in the leukocytic differential count.[110] The small number of persons with this disease studied and the lack of observations concerning the degree of arterial oxygen saturation prevent, however, an adequate comparative study.

COMMENT

Attention has been called by many investigators to the wide individual variations observed in the hematologic response to the anoxemia associated with exposure to a low pressure environment. This characteristic has also been noted in the present study; however, an analysis of the collected data indicates that in general the level of polycythemia, together with the signs of erythropoietic hyperactivity and increased amounts of serum bilirubin, are related to the degree, duration and continuity of the anoxic stimulus and that the variability affects chiefly the early phase of the response (table *27*). When the period of anoxemia is short, of a few hours' duration, the increase in red blood cells and hemoglobin is not a constant phenomenon and, when it occurs, shows the individual variations which have been repeatedly emphasized. On the other hand, when the anoxemia is permanent, there is found in a large majority of cases of polycythemia the level of which is closely related to the degree of anoxic stimulus. Intermittent exposure to anoxemia occupies an intermediate position from the point of view of the hematologic response; the occurrence of polycythemia and its level, though there are large variations in the latter, are also broadly related to the frequency and intensity of the anoxic stimulus.

It appears that in the presence of an anoxic condition the release of stored red blood cells is a labile and variable emergency mechanism of adaptation; the late response, based on an increased production of cells by a hyperactive bone marrow, is a more constant adaptative process. Many of the apparently contradictory results obtained in hematologic studies made at high altitudes and in low pressure chambers seem to be related to different experimental conditions in regard to the duration of the exposure and the level of altitude, or simulated altitude, reached.

The more or less definite inverse relationship which exists between the level of polycythemia and the degree of arterial oxygen saturation in dwellers at high altitudes, corroborates the opinion advanced by Bert,[1] Viault [2] and Miescher [111] in the latter part of the last century, and accepted by most investigators, that the deficient oxygen supply to the bone marrow is the main stimulus for the increased production of red blood cells and hemoglobin. The fact that at the same altitude the polycythemia is more pronounced in men with a higher degree of arterial oxygen unsaturation (as in the subjects with silicosis studied by us) indicates that environmental factors, such as increased solar radiation, do not play a fundamental role in the causative mechanism of the hematic response as was suggested by Kestner.[112] While it has been generally accepted that at high altitudes and in other similar conditions the low tension of the oxygen

108. Minot, G. R., and Buckman, T. E.: Am. J. M. Sc. **166**:469, 1923. Harrop and Wintrobe.[89]

109. Tinney, W. S.; Hall, B. E., and Giffin, H. Z.: Proc. Staff Meet., Mayo Clin **18**:46, 1943.

110. Nadler, S. B., and Cohn, I.: Am. J. M. Sc. **198**:41, 1939.

111. Miescher, F.: Cor.-Bl. f. schweiz. Aertze **23**: 809, 1893.

112. Kestner, O.: Ztschr. f. Biol. **73**:1, 1921.

physically dissolved in the plasma constitutes the immediate stimulus for the erythropoietic hyperactivity, the relative importance of other factors, such as the percentage of hemoglobin saturation and the absolute concentrations of reduced and oxygenated hemoglobin, remain yet to be adequately understood; in anemic anoxia, clinical or experimentally produced, in which the blood oxygen tension is normal, a relationship has been noted to exist between the degree of erythropoietic hyperactivity and the level of anemia.[113] There seems to be a limit for the anoxic factor in the stimulation of the erythropoietic activity; when the arterial oxygen saturation reaches a value of about 60 to 70 per cent, there is a decrease, rather than a further increase, in the hemoglobin and red cell count. This suggests that the hematologic compensatory adjustment ceases to be effective at a degree of anoxemia which corresponds, roughly, to an altitude of 6,000 meters, or 20,000 feet; above this level the proportion of reduced over oxygenated hemoglobin increases rapidly. This limit in the polycythemic response does not appear to be related to an anatomic limitation of the erythropoietic organs, because no plateau, or flattening, is observed in the curve representing the level of polycythemia with increasing degrees of anoxemia. It may be associated with a higher rate of destruction of cells, as has been suggested by Talbott[56]; the accentuation of reticulocytosis, which has been observed to occur with a high degree of anoxemia would favor this interpretation, but on the other hand this is not accompanied with an increased accumulation of bilirubin in the plasma. According to our observations, an increasing degree of anoxemia has a tendency to lower the hemoglobin concentration of the circulating erythrocytes; this fact makes it seem possible that an interference with the production of hemoglobin rather than with the building of the erythrocytic stroma is responsible for the lack of an appropriate polycythemic response to severe degrees of anoxemia.

From the observations made in men constantly exposed to a low barometric pressure it appears that the level of hemoglobin in the circulating blood can be regarded as one of the many factors which tend to preserve the internal stability, or homeostasis, as it has been called by Cannon,[114] against the disturbing influence of a low oxygen tension in the inspired air; it increases, up to a certain limit, in relation to the degree of disturbance in the external environment and decreases when normal conditions are restored. It is interesting to observe that chronic anoxemia, even when present since birth, does not modify permanently the activity of the erythropoietic organs. When the native of high altitudes comes down to sea level his blood acquires, after a certain time, the same morphologic characteristics as those found in persons who were born and have lived at sea level. This fact eliminates the possibility that racial factors play a part in the development of polycythemia at high altitudes. The frequent moderate reduction in red blood cells and hemoglobin which has been observed in men born at high altitudes during their first few months of residency at sea level may be related to an adaptative period on the part of the bone marrow to the disappearance of the potent anoxic stimulus present at high altitudes. These findings are not unlike those of Gordon and Kleinberg,[115] who observed in animals a moderate process of anemia after cessation of exposure to a low pressure in a chamber.

The polycythemia of high altitudes, which has been classified as an erythrocytosis, is, according to this and previous studies, absolute in type. An increase in the circulating blood volume, due to a larger cell mass with an unchanged or decreased plasma volume, has been observed in persons who have just arrived at high altitudes and in healthy and in diseased human beings and animals living permanently in such environment. The increase tends to be proportional to the level of altitude and the degree of anoxemia, often reaching high values. It appears that there must be a modification of the following concept, advanced about fifteen years ago[86d] and still found in the literature: "Increases in blood volume to 115 or 120 cubic centimeters for each kilogram of body weight, rarely or never occur as responses of the blood to lowered oxygen tension." A slight macrocytosis is another characteristic of the polycythemia related to a constant exposure to a low barometric pressure. The increased size of the red blood cells may be due, in part, to the erythropoietic hyperactivity[116] and to the presence of an excess of reticulocytes, cells which have been found to have a large volume.[117] Whether this morphologic variation, which includes a greater corpuscular surface area, represents a compensatory adjustment on the part of the circulating respiratory unit for the acquisition and transport of the oxygen is an interesting possibility. The stimulating influence of the anoxemia caused by a low oxygen tension in

113. Robscheit-Robbins, F. S., and Whipple, G. H.: Am. J. Physiol. **134**:263, 1941. Hurtado, Pons and Merino.[19]

114. Cannon, W. B.: Physiological Regulation of Normal States: Some Tentative Postulates Concerning Biological Homeostatics, in Jubilee Volume for Charles Richet, Paris, 1926, p. 91.

115. Gordon, A. S., and Kleinberg, W.: Proc. Soc. Exper. Biol. & Med. **37**:507, 1937.

116. Haden, R. L.: J. Lab. & Clin. Med. **22**:439, 1937.

117. Cruz, W. O.: Am. J. M. Sc. **202**:781, 1941.

the inspired air is restricted to the processes concerned with the production of red blood cells and hemoglobin. Leukogenetic activity remains undisturbed even in persons who have a high level of polycythemia and a severe degree of anoxemia. Only during the early period of exposure to a low barometric pressure is observed, occasionally and with more frequency at the higher altitudes, a moderate and temporary leukocytosis, probably related to factors of mobilization and release. The tendency for an inversion in the leukocytic formula, with a relative increase in the lymphocytes and a proportional reduction in the other cells, observed in dwellers at high altitudes, even at relatively low elevations, has been ascribed to the action of a more intense solar radiation.[118]

The polycythemia which occurs under the influence of a temporary anoxemia, such as the one observed on persons' arrival at high altitudes, seems to be in most cases the combined result of release or mobilization of stored blood and a process of hemoconcentration. The comparative study of the values for blood volume determined for the same subject within the first two hours after his arrival at high altitudes and a few days previously is open to criticism on account of the possible physiologic variations which might have taken place during the interval, but the constancy of the results obtained suggests, however, that the mechanisms mentioned play a part in the observed polycythemia. The existence of reserves of blood in man, almost universally accepted since the classic experimental work of Barcroft[119] and others on the role of the spleen, has been recently denied by Ebert and Stead,[120] who investigated the hematic response to exercise, epinephrine and hemorrhage, employing the blood volume method of Gibson and Evans.[15] But the observations of Kaltreider, Meneely and Allen[121] on the effect of epinephrine on the volume of blood and those of Glickman and others[122] concerning the effect of hot environmental conditions, made by the same dye method, and the recent studies of Watson and Paine,[123] who analyzed the blood obtained directly from the splenic vein after the injection of epinephrine into the splenic artery, give fairly good evidence that reserves of blood do exist in the body.

The polycythemia of chronic anoxemia corresponds to an increased erythropoietic activity, as evidenced by the excess of reticulocytes found in the peripheral blood. This reticulocytosis, which is more pronounced the higher the altitude and the degree of anoxemia, cannot be explained simply on the basis of a greater number of red blood cells per cubic millimeter; it is a qualitative as well as a quantitative process, and of a given number of erythrocytes expelled by the bone marrow a higher proportion are immature at high altitudes than at sea level. The absence of nucleated red cells in the peripheral blood suggests that the increased erythropoietic activity proceeds in a somewhat orderly fashion.

A definite elevation of the serum bilirubin, due almost entirely to an increase in the indirect fraction, is another fairly constant finding in men exposed frequently or permanently to a low barometric pressure. There are wide individual variations, but in general the increase in the pigment is directly proportional to the level of polycythemia (table 27), which suggests that a greater rate of cellular destruction, which corresponds, in turn, to an increased rate of formation, is an important factor in the mechanism causing the hyperbilirubinemia. Whether the normal liver cells should be able to excrete all the pigment, regardless of its rate and amount of formation, is still an unsettled question among students of hepatic physiology. There are other factors present at high altitudes which may decrease the efficiency of this organ in its excretory function. The well known investigations of Rich,[124] who observed that rats placed in low oxygen tension chambers showed a diminished ability to excrete intravenously injected bilirubin and had degenerative changes in the liver cells, and the similar findings of Campbell,[125] in regard to organic changes that developed in other animals placed under similar experimental conditions, suggest that anoxemia per se when prolonged or constantly present may be an important factor in causing hepatic insufficiency from the point of view of excretion of pigment. Our findings of a proportional relationship between (1) the level of hyperbilirubinemia and the degree and duration of the anoxemia and (2) the abnormal retention of injected bilirubin in native residents of high altitudes are in favor of that possibility. The increase in the circulating blood volume, a characteristic of the polycythemia of high altitudes, may play an additional role in the

118. Kennedy, W. P., and Mackay, I.: J. Physiol. **87**:337, 1936.

119. Barcroft, J.: Lancet **1**:319, 1925.

120. Ebert, R. V., and Stead, E. A.: Am. J. M. Sc. **201**:665, 1941.

121. Kaltreider, N. L.; Meneely, R., and Allen, J. D.: J. Clin. Investigation **21**:339, 1942.

122. Glickman, N.; Hick, F. K.; Keeton, R. W., and Montgomery, M. M.: Am. J. Physiol. **134**:165, 1941.

123. Watson, C. J., and Paine, J. R.: Am. J. M. Sc. **205**:493, 1943.

124. Rich, A. R.: Bull. Johns Hopkins Hosp. **47**: 338, 1930.

125. Campbell, J. A.: Lancet **2**:84, 1928.

etiologic mechanism of the hyperbilirubinemia, according to recent studies made in cases of polycythemia vera by Tinney, Hall and Giffin;[109] these investigators found hepatic complications in 25 per cent of 163 cases of the latter disease, and suggested that these may be due to several factors, among which they mentioned distention of the portal circulation in the liver, stasis caused by increased viscosity of the blood and impairment of the nutrition of the hepatic cells as a result of the circulatory alterations. Urteaga[126] has suggested that a raised threshold of the hepatic cells for excretion of bilirubin may be present at high altitudes. Further studies are necessary for the final elucidation of the problem, but it appears likely at the present time that the hyperbilirubinemia present in most persons exposed frequently or constantly to a high altitude is the combined effect of several factors, among which an increased rate of pigment formation and a decreased power of the hepatic cells to excrete pigment, due to the anoxemia, are probably the most important. Another aspect, related to the possible increased rate of cellular destruction present at high altitudes, is the possibility that the products derived from the red cell disintegration will serve as an additional stimulus for increased formation, as has been observed experimentally.[127] If so, the person subjected to the influence of chronic anoxemia would be placed in a sort of a vicious circle in regard to erythropoietic activity.

The effectiveness which the polycythemia of high altitudes has as a compensatory process is still a debated question. There is abundant evidence to show that the development of symptoms associated with exposure to a condition of temporary anoxemia and the degree of acclimatization found in men living permanently at high altitudes cannot be strictly rated in terms of polycythemic levels. The understanding of these aspects becomes clearer if one realizes that the essential problem at high altitudes is not limited to the amount of oxygen carried in the blood; as a matter of fact, as it was originally pointed out by Barcroft and others,[8] the resident at a high altitude has in his arterial blood a higher oxygen content than the person living at sea level. The chief difficulty lies in the reduced tension of the small fraction of gas physically dissolved in the plasma, which represents the immediate source of supply to the tissues; a high hemoglobin concentration with a high oxygen content in the circulating blood, although decreasing, in part, the effects of the reduced tension, is not, in consequence, incompatible with all the limitations which an exposure to a low barometric pressure brings to the body. A significant observation has been recently made by Dorrance and others[128]; these investigators observed that the polycythemia caused by the administration of cobalt to rats increased the work performance of these animals under conditions of anoxia. The resident of high altitudes has been almost exclusively studied at rest; his capacity for graded physical activity and the relative importance of his various organic and functional characteristics in the adaptation to the increased demands of work are practically unknown.

Further studies concerning muscle hemoglobin, which has been found to be increased in dogs living at high altitudes[129] but has not yet been investigated in human beings, will be of considerable interest from the point of view of acclimatization. Owing to its peculiar characteristics in regard to the affinity and combination with oxygen,[130] this substance may play an important role in compensatory adjustment to a deficient oxygen supply, as has been already suggested.[131]

The study of the hematologic observations made by several investigators at sea level on persons with some degree of arterial oxygen unsaturation due to pulmonary disease shows that the polycythemic response in them was less constant and in general less pronounced than that observed by us in men living at high altitudes with a similar degree of anoxemia, except in cases of Ayerza's disease. No adequate explanation can be given at the present time for this difference. The lack of polycythemia is especially frequent in cases of pulmonary emphysema, in spite of the almost constant anoxemia present in this disease. It suggests that, in addition to the fundamental anoxic stimulus, some other factors, perhaps associated with the nature of the pulmonary lesions, circulatory abnormalities, reflex mechanism, etc. may play some secondary role in bringing on the erythropoietic hyperactivity. It is interesting in this respect that the persons with Ayerza's disease, in whom the associated polycythemia usually reaches high values, have clinical and hematologic characteristics (appearance of the roentgenograms of the lungs, abnormal lowering of the arterial oxygen

126. Urteaga, O.: An. Fac. de med., Lima **25**:89, 1942.

127. Boycott, A. E., and Oakley, C. L.: J. Path. & Bact. **36**:205, 1933.

128. Dorrance, S. S.; Thorn, G. W.; Clinton, M.; Edmonds, H. W., and Farber, S.: Am. J. Physiol. **139**:399, 1943.

129. Hurtado, A.; Rotta, A.; Merino, C., and Pons, J.: Am. J. M. Sc. **194**:708, 1937.

130. Millikan, G. A.: J. Physiol. **87**:38P, 1936.

131. Hill, R.: Proc. Roy. Soc., London, s.B **120**: 472, 1936.

saturation, right-sided heart failure, etc.) similar to those of patients with chronic mountain sickness, who at high altitudes show an abnormal accentuation of the polycythemia.[75]

An increase in the circulating blood volume is a common finding in the polycythemia of high altitudes and in polycythemia vera, but a comparative study of these processes raises a strong argument against the acceptance of the theory that an anoxic stimulus plays a significant role in the development of the latter disease. The increase in the red cell volume tends to be greater in polycythemia vera, which also lacks the proportional relationship between the level of polycythemia and the signs of increased red cell formation and destruction observed in men exposed constantly to a low oxygen tension. The absence of nucleated red cells in the peripheral blood (a not infrequent finding in polycythemia vera) at high altitudes, regardless of the degree of anoxemia and the level of polycythemia, and, of fundamental importance, the lack of disturbance in the leukogenetic activity indicates that the hematologic response to anoxia does not show a disorder of the entire hemopoietic system, such as occurs often in polycythemia vera. An apparently greater similarity, from the point of view of the increase in the circulating blood volume, is found between this disease and chronic mountain sickness, but the fundamental differences, in the characteristics of the bone marrow activity and the involvement of the leukocytes are also evident in these processes. In addition, there is some evidence that the hematologic alterations in chronic mountain sickness are related to pulmonary factors not present in polycythemia vera.[75]

SUMMARY AND CONCLUSIONS

Investigations have been made, at sea level and at high altitudes, in several series of healthy and diseased male subjects, concerning the influence of temporary, intermittent and chronic anoxic anoxia (anoxemia) on the morphologic and other characteristics of the circulating blood. The related literature has been briefly reviewed. The main observations lead to the following conclusions:

1. Exposure to a low barometric pressure environment causes in most cases a polycythemic response. There are wide individual variations, but in general the level of polycythemia is directly proportional to the degree, duration and continuity of the anoxic stimulus.

2. There seems to be a limit for the hematologic response to the anoxic stimulus. When this is extremely severe, a decrease, rather than a further increase, is observed in the resulting polycythemia. An interference with the formation of hemoglobin may be the responsible mechanism.

3. The level of hemoglobin in the circulating blood may be considered as one of the many factors which tend to preserve the internal stability, or homeostasis, against the disturbing influence of a constant lowering of the oxygen tension in the inspired air.

4. The polycythemia associated with the anoxemia of high altitudes is absolute in type. The elevation in the total blood volume, which at times reaches high values, is due to an increased red cell volume.

5. The polycythemia observed on persons' arrival at high altitudes seems to be due to factors of release of stored blood and hemoconcentration; that corresponding to a repeated or constant exposure to a low pressure environment is related to an erythropoietic hyperactivity.

6. The polycythemia associated with a constant or intermittent anoxemia tends to show a proportional elevation in the circulating reticulocytes and in the serum bilirubin. The latter characteristic suggests that an increased rate of cellular destruction parallels the increased formation, but other factors, such as insufficiency of the liver in excretion of pigment, due to the anoxic condition, may also play an etiologic role in the observed hyperbilirubinemia.

7. The stimulating influence of anoxemia on the hemopoietic system is restricted to the formation of red blood cells and hemoglobin. Leukopoietic activity is not affected, and the moderate and temporary leukocytosis which at times is observed on a person's arrival at a high altitude is probably related to the release and mobilization of stored blood.

8. Chronic anoxemia does not modify the erythropoietic activity permanently. When a person who has lived since birth at high altitudes is brought down to sea level, he shows after some time blood characteristics similar to those found in persons who have always lived under the latter environmental conditions. During the early period of adaptation to the normal pressure environment there frequently occurs an abnormal decrease in the red blood cells and hemoglobin.

9. Comparative study of the polycythemia of high altitudes and the polycythemic processes observed at sea level indicates that: (*a*) in cases of anoxemia at sea level due to pulmonary changes, the polycythemic response tends to be less than with corresponding degrees of arterial oxygen unsaturation at high altitudes, except in cases of Ayerza's disease; (*b*) it is not likely that the causative mechanism of polycythemia vera is related to the existence of an anoxic stimulus.

EFFECTS OF SOME LIVER EXTRACTS ON THE CARBOHYDRATE METABOLISM

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The nineteen-twenties will go down in medical history as the decade of two great therapeutic discoveries. Barely four years had elapsed since Banting's epochal discovery of insulin when Minot and Murphy [1] startled the world by their announcement of the effect of liver on the course of pernicious anemia. The groundwork for this discovery was laid earlier by the studies of Whipple and his associates [2] on the effect of a diet rich in red meat and liver on erythrocytopoietic activity in dogs with experimentally produced anemia.

The next important step in the progress of liver therapy was the preparation of an active liver extract which could be administered either by mouth or parenterally. This overcame the objections of many patients to the monotony of a daily diet including liver.

With the value of liver therapy firmly established, two questions occupied the minds of investigators: 1. What is the nature of the antianemia principle present in the liver? 2. What side effects, if any, do liver or liver preparations have on other functions of the body? This paper is concerned only with the second question.

That liver and liver extract influenced the carbohydrate metabolism was claimed long before the discovery of insulin and the introduction of liver as a specific treatment for pernicious anemia. At the end of the nineteenth century French clinicians advocated the use of liver and of liver extracts in the treatment of diabetes mellitus. The results, however, were not uniform. Sometimes the administration of liver or of liver extract was followed by a remarkable improvement. The glycosuria seemed to be diminishing or disappeared entirely, and the patient felt better. At other times liver had no effect on the course of the disease; on the contrary, the diabetic symptoms increased in severity, with a corresponding increase in the urinary concentration of dextrose.

To explain these divergent results obtained with liver therapy Gilbert and Carnot [3] assumed that a diabetic patient might suffer from either hyperfunction or hypofunction of the liver. Thus, they reasoned, a person with diabetes and a hypofunctioning liver will ultimately benefit from the liver diet, while a patient with a hyperfunctioning liver will get worse. In their conclusion they went one step further, advocating the use of a liver diet as a test of the functional activity of the liver in the diabetic person. Other French clinicians published similar reports on the effect of liver in diabetes. Even as late as 1921, attempts were still being made to treat diabetes with liver extract. In that year Levin,[4] in an article entitled "My Observations With Hepatic Extract As a Remedial Agent," related the good results he obtained with a specially prepared soluble liver extract in 4 diabetic patients. With the introduction of insulin as a standard treatment of diabetes these experiments were abandoned.

The publication of an article by Blotner and Murphy [5] in which they claimed for liver an insulin-like action on the blood sugar concentration both in diabetic and in nondiabetic persons brought the whole question of the effect of liver on the carbohydrate metabolism back into the limelight. In a second paper [6] they described the results obtained from the administration of a specially prepared liver extract in 19 diabetic patients. Their conclusions were similar to those already reported, namely, that their liver extract contained a sugar-reducing substance, which was active when taken by mouth, was nontoxic and had an effect on the blood sugar concentration similar to that obtained with small doses of insulin. A similar report was published by Lombardi.[7] On the other hand, Brett and

1. Minot, G. R., and Murphy, W. P.: Treatment of Pernicious Anemia by Special Diet, J. A. M. A. **87**:470 (Aug. 14) 1926.

2. Whipple, G. H., and Robscheit-Robbins, F. S.: Am. J. Physiol. **72**:408, 1925.

3. Gilbert and Carnot: Gaz. d. hop. **73**:995, 1900.

4. Levin, A. L.: South. M. J. **15**:175, 1922.

5. Blotner, H., and Murphy, W. P.: Effect of Liver on Blood Sugar Level, J. A. M. A. **92**:1332 (April 20) 1929.

6. Blotner, H., and Murphy, W. P.: Effect of Certain Liver Extracts on Blood Sugar of Diabetic Patients, J. A. M. A. **94**:1811 (June 7) 1930.

7. Lombardi, E.: Riforma med. **46**:7, 1930.

his associates,[8] working with a liver extract carefully prepared according to the recommendations of Blotner and Murphy, not only failed to substantiate these claims but, on the contrary, noticed a slight rise in the blood sugar concentration following the administration of the liver extract.

An interesting report on the comparative effects of liver and of liver extract in pernicious anemia and diabetes, in one of those rare cases in which the patient suffered from both diseases, was made by Bowen.[9] Bowen's patient, a man 55 years old, was found to have pernicious anemia in 1928. He was then treated with raw liver and liver extract. In March 1929 he began to show symptoms of diabetes. He was therefore given insulin for the diabetes in addition to whole liver for the anemia. During this period the amount of insulin had to be reduced on account of the ensuing hypoglycemia. At this stage liver extract was substituted for whole liver. During the latter period the amount of insulin had to be doubled to control the diabetic state. In this case, judging from the clinical progress of the patient, the liver extract did not lower the blood sugar concentration; on the contrary, the amount of insulin had to be doubled to control the diabetes.

In connection with the question of the effect of liver extract on the carbohydrate metabolism, Gensslen's[10] report seems to throw some light on the probable mode of action of some liver extracts. He gave normal rabbits injections of a potent liver extract and found that after injection of 0.5 cc. of the extract daily for several days the animal's hepatic glycogen, which normally ranges between 4 and 6 per cent, practically disappeared. From this experiment it would appear that this liver extract had a glycogenolytic effect on the hepatic glycogen.

Buttner[11] conducted similar experiments with a protein-free liver extract. Seven out of 9 patients who were given injections of 2 cc. of this extract showed increases in the blood sugar averaging 33 per cent, the maximum increase running as high as 60 per cent. Similar results were obtained by the same author in experiments with normal rabbits. To exclude the influence of the adrenal glands he repeated these experiments with adrenalectomized and morphine-poisoned animals. The results, however, remained the same.

My own experience with the effect of liver extracts on the carbohydrate metabolism goes back to the years 1932 and 1933. During this period 17 patients, all suffering from pernicious anemia, were treated by me with liver extract[12] for various lengths of time. The routine treatment consisted of intramuscular injections of liver extract given twice weekly. The extract was intended for parenteral administration and was delivered in individual ampules containing about 5 cc. of the extract. I used two fractions of the extract; they were labeled extract fraction A and extract fraction B. To test the potency of these fractions the patients were divided into two groups. One group received regularly the fraction A extract, while the other group was treated with the fraction B extract. The hematologic response to both extracts was good.

REPORT OF CASES

Case 1.—A woman in her late fifties who was treated with the fraction B extract soon began to complain of a severe itching in the vulva. A determination of the fasting blood sugar and an examination of the urine were therefore made. The urine gave a positive reaction for sugar with Benedict's reagent, and the blood sugar was reported to be at 150 mg. per hundred cubic centimeters. A month later another determination of the blood sugar was made. This time the value rose to 190 mg. The patient was then referred to the clinic for diabetic patients, and the antianemia treatment with the extract was continued. Six weeks later the blood sugar was found to have reached a concentration of 240 mg. per hundred cubic centimeters. Since the coexistence of diabetes and pernicious anemia was always regarded as extremely rare, this case aroused my interest. In investigating the case it was important to establish whether the diabetes preceded the anemia or developed after it. This could easily be established since the patient had been referred to the clinic from the ward. The hospital record showed that about six months previously the blood sugar was 90 mg. per hundred cubic centimeters and the urine was normal.

The assumption that the liver extract might in some way be connected with the development of the diabetes, especially after consideration of the steady and rapid rise in the blood sugar concentration, could not easily be dismissed. To make sure that this was not a mere coincidence, I examined the urine and the blood of several other patients who had been treated with the same extract. The results of these examinations confirmed my suspicions. All had hyperglycemia and glycosuria.

Case 2.—M. C., a man about 60 years old, had suffered from pernicious anemia for some years. On a recent admission to the hospital his blood sugar was found to be 110 mg. per hundred cubic centimeters and his urine was free of sugar. After four weeks of treatment with the fraction B extract, his blood sugar was 140 mg. per hundred cubic centimeters and his urine gave a positive reaction for sugar with Benedict's

8. Brett, P.; Broom, W. A., and Howitt, F. O.: Lancet **1**:20, 1931.

9. Bowen, B. D.: Comparative Effect of Liver and Liver Extract on Diabetes in Case of Combined Pernicious Anemia and Diabetes, J. A. M. A. **95**:30 (July 5) 1930.

10. Gensslen, M.: Klin. Wchnschr. **9**:2099, 1930.

11. Buttner, H. E.: Klin. Wchnschr. **11**:1218, 1932.

12. The extract was supplied by the Wilson Laboratories, Chicago; 1 Gm. of the extract represented 25 Gm. of fresh liver.

reagent. Four weeks later the blood contained 190 mg. of dextrose per hundred cubic centimeters. Six weeks later, with the patient still under treatment with fraction B extract, the blood sugar reached a level of 240 mg. Several weeks later it rose to 320 mg.

CASE 3.—Another patient in this group, E. S., a man, whose blood sugar had been found to be normal about a year previously, had values ranging between 190 and 300 mg. per hundred cubic centimeters of blood after several months of treatment with the fraction B extract.

COMMENT

The remaining 5 patients of this group reacted in the same way to the administration of the extract. All had dextrose in the urine and hyperglycemia in some degree. In several instances I was able to determine the blood sugar concentration just before the treatment with this extract was started. I found that after six to eight injections of liver extract fraction B all patients had a 40 to 50 per cent rise in the blood sugar concentration, and 1 patient had glycosuria.

Fasting Blood Sugar Values and Urinary Reactions of Seventeen Patients with Pernicious Anemia Treated with An Injectable Liver Extract, Fraction A or B

No.	Name	Sex	Fasting Blood Sugar, Mg. per 100 Cc. of Blood: Before Liver Treatment	Fasting Blood Sugar, Mg. per 100 Cc. of Blood: During Liver Treatment	Urine
1	M. C.	M	110	140-320	Glycosuria
2	E. S.	M	?	180-300	Glycosuria
3	N. A.	F	90	150-240	Glycosuria
4	R. S.	F	?	190	Glycosuria
5	C. R.	F	90	190	Glycosuria
6	M. R.	F	?	140-160	Glycosuria
7	R. K.	F	?	140-160	Glycosuria
8	N. G.	M	?	140-160	Glycosuria
9	A. N.	M	120	150	No sugar
10	S. W.	M	100	150	No sugar
11	P. C.	F	95	140	No sugar
12	H. H.	F	100	90	No sugar
13	H. D.	M	120	140	No sugar
14	M. T.	F	100	105	No sugar
15	M. D.	M	?	90	No sugar
16	H. G.	M	?	105	No sugar
17	C. S.	F	?	120	No sugar

Further observation of these patients could not be made by me because of my resignation from the clinic. However, the attending physician, who is still in charge of the clinic, was kind enough to give me some later information about these patients. In a letter addressed to me in 1942 he wrote: "Some of the cases you worked on have been established definitely now as instances of diabetes, and the hyperglycemia may not necessarily have developed as a result of the liver extract."

Personally, I have no doubt that the liver extract was responsible for the development of the diabetes. There is enough clinical and laboratory evidence today to support this statement.

The experiments conducted by Dr. Joseph E. Sokal in Dr. Long's laboratories proved conclusively the presence of a glycogenolytic substance in the liver extract I used in the clinic for the treatment of patients with pernicious anemia. This extract when injected into normal rabbits regularly produced glycosuria and hyperglycemia.

SUMMARY

A liver extract [12] used in the treatment of patients with pernicious anemia was found to have an effect on the carbohydrate metabolism. Of the 17 patients treated with this liver extract, fraction A or B, 11 had an increase in the blood sugar concentration, 8 had glycosuria and hyperglycemia in various degrees, depending on the length of treatment, and the remaining 6 were unaffected. Some of the patients became permanently diabetic.

CONCLUSIONS

1. Judging from clinical observations and experimental work in the laboratory, it appears that some liver extracts used in the treatment of pernicious anemia contain an as yet unknown factor, not identical with the antianemia principle, which is capable of producing glycosuria and hyperglycemia in human beings as well as in experimental animals.

2. All liver extracts before being released for public use should be tested for the presence of this "diabetogenic" factor.

3. Extracts possessing such properties could be used effectively in the treatment of chronic hypoglycemia.

4. Periodic determinations of blood sugar should be made for all patients treated with liver extract.

EFFECTS OF SOME LIVER EXTRACTS ON CARBOHYDRATE METABOLISM

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INTRODUCTION AND REVIEW OF THE LITERATURE

The announcement by Minot and Murphy of the successful therapy of pernicious anemia by a liver diet served as a strong stimulus to the preparation and study of liver extracts. Although the primary interest of investigators has been in the effect of various liver extracts on the hemopoietic system and most of the studies have been confined to patients with pernicious anemia, there has been some consideration of the action of such extracts on other organ systems. With the introduction of injectable extracts in particular, animal experiments have been performed and various side actions of liver preparations have been studied.

In the course of such studies of the systemic effects of orally and parenterally administered liver extracts, especially the latter, various observers have noted more or less striking effects on the carbohydrate equilibrium of the body. As might be expected with different men using various technics and working with extracts prepared often by widely divergent methods, there is no agreement in the reported results. Thus one finds papers denying any effect of liver extract on carbohydrate metabolism, opposed by reports of clearcut and definitely established actions, in opposing directions.

Because of the contradictory nature of the findings and because much of the investigation has been poorly controlled, accurate appraisal and evaluation of the published work are difficult. The reports in the literature fall into several groups.

Effect of Liver Extract on Blood Sugar in Experimental Animals.—Büttner [1] found that both of two commercial extracts tested produced regularly a more or less distinct rise in the blood sugar of fasting and fed rabbits. In the latter, after feeding of levulose increases of more than 150 mg. per hundred cubic centimeters above the values for the control animals were noted. The blood sugar–raising effect was noted in adrenalectomized and in morphine-poisoned rabbits. Kähler and Riedel [2] were unable to confirm Büttner's results. However, the latter [3] raised certain valid objections against the experimental technic and dosage that they used. Zipf and Dringenberg [4] also were unable to confirm Büttner's findings. They examined four liver preparations, including the two used by Büttner, and obtained no consistent effect on the fasting blood sugar, alimentary hyperglycemia or insulin hypoglycemia of rabbits. On the other hand, Hungerland [5] noted that daily injections of liver extract into rabbits for four to seven weeks produced a resistance to insulin and a rapid recovery from insulin hypoglycemia as compared with the course in controls.

Effect of Liver Extract on Glycogen Content of Liver.—Gaensslen [6] noted that injection of his liver extract into guinea pigs caused a prompt and remarkable drop in hepatic glycogen. He obtained values ranging from a trace to 1.5 per cent, as against 4.8 per cent and 5 per cent in control animals. Ederle and Kriech [7] also noted a glycogenolytic effect of liver extract and ascribed it to the protein content of the extracts used. De Caro [8] reported that administration of

From the Department of Physiological Chemistry, Yale University Medical School.

Dr. C. N. H. Long and Miss Edith Fry gave advice and criticism during the studies reported and made laboratory facilities available.

1. Büttner, H. E.: Clinical and Experimental Observations on the Action of Injectable Liver Extracts, Klin. Wchnschr. **11**:1218, 1932.

2. Kähler, H., and Riedel, R.: Does Liver Extract Exert an Effect on Blood Sugar? Klin. Wchnschr. **12**: 151, 1933.

3. Büttner, H. E.: Liver Extracts: Effect on Blood Sugar, Klin. Wchnschr. **12**:152, 1933.

4. Zipf, K., and Dringenberg, H.: The Effect of Liver Extract on Carbohydrate Metabolism, Arch. f. exper. Path. u. Pharmakol. **170**:465, 1933.

5. Hungerland, H.: The Effect of Liver Extract on Insulin Hypoglycemia, Klin. Wchnschr. **18**:647, 1939.

6. Gaensslen, M.: A Highly Active Injectable Liver Extract, Klin. Wchnschr. **9**:2099, 1930.

7. Ederle, W., and Kriech, H.: Hepatic Glycogenolytic Action of Organ Extracts Containing Protein, Med. Welt **6**:184, 1932.

8. De Caro, L.: The Action of Liver Extract on Hepatic Glycogen and Basal Metabolism, Boll. Soc. ital. di biol. sper. **7**:344, 1932.

liver extract caused a decrease in hepatic glycogen in the rat and guinea pig, without affecting blood sugar or basal metabolism. On the other hand, Wiedemann [9] found that liver extract caused a rise in hepatic glycogen. He obtained values as high as 11.3 per cent in normal guinea pigs.

Effect of Liver Extract on Blood Sugar of Human Subjects.—The reports by Blotner and Murphy [10] that oral administration of liver extract had a favorable effect on the blood sugar of diabetic patients aroused much interest. However, later careful work with clinical material and depancreatized dogs (De Pencier, Soskin and Best [11]) failed to confirm these startling findings. There are several instances on record in which hyperglycemia and glycosuria have been observed in the course of treatment of patients with pernicious anemia with crude liver extract. The most striking of these is the experience of H. B. Sokal,[12] who found that hyperglycemia developed in 11 of 17 patients treated with a crude liver extract and that 8 of them had glycosuria.

The striking values for liver glycogen obtained by Gaensslen and those for blood sugar obtained by H. B. Sokal after parenteral administration of liver extract prompted this study of the effect of liver extract on certain aspects of carbohydrate metabolism. Aims of the work reported herein were (*a*) to confirm if possible the results described by these investigators, (*b*) to study the mechanism involved and (*c*) to investigate the properties of the fraction responsible (it seemed most probable that the carbohydrate-affecting fraction was independent of the antianemic factor).

Several liver extracts were available for study, among them three similar to those used by H. B. Sokal. Unfortunately, extracts used by other authors cited could not be obtained. It was most feasible to use the albino rat as the experimental animal.

9. Wiedemann, A.: Effect of Liver Extract on the Carbohydrate Metabolism of the Liver, Klin. Wchnschr. **17**:766, 1938.

10. Blotner, H., and Murphy, W. P.: Effect of Certain Liver Extracts on the Blood Sugar of Diabetic Patients, J. A. M. A. **94**:1811 (June 7) 1930.

11. De Pencier, M. T.; Soskin, S., and Best, C. H.: The Effect of Liver on the Blood Sugar Level and on the Sugar Excretion of Depancreatized Dogs, Am. J. Physiol. **94**:548, 1934.

12. Sokal, H. B.: Does Liver Extract Contain a Diabetogenic Substance? Report of Eight Cases of Pernicious Anemia with Hyperglycemia and Glycosuria, unpublished data; personal communication to the author (1934).

PLAN OF EXPERIMENTS

Preliminary experiments included brief examination of the liver extracts available, selection of a standard dilution of the concentrate in which the bulk of the extract used was supplied, selection of experimental animals and standardization of technic. Investigation then proceeded along the following lines:

I. Daily determination of urinary dextrose over long intervals, both during administration of liver extract and during control periods

- A. In normal animals
- B. In "latent" diabetic (partially depancreatized) animals
- C. In a "frankly" diabetic (partially depancreatized) animal excreting large amounts of dextrose daily

II. Effect of parenteral liver extract on blood dextrose levels of normal animals

- A. In the fasting state
- B. In the absorptive and postabsorptive states

III. Effect of insulin on hyperglycemia produced by liver extract

IV. Effect of liver extract on carbohydrate stores of normal animals

- A. Fasting
- B. Fed

V. Effect of parenteral liver extract on nitrogen balance of normal animals

VI. Effect of parenteral liver extract on respiratory quotients of

- A. Normal animals
  1. Fasting
  2. During absorption of dextrose
- B. "Diabetic" (partially depancreatized) animals
  1. Fasting
  2. During absorption of dextrose

Finally, some properties of the active principle were studied, and attempts were made to fractionate the liver extract used. Postmortem studies and microscopic examination of representative tissue sections were carried out routinely, all animals being killed at the conclusion of the experiments.

METHODS

Care of Animals.—The animals were housed in a warm room, free from drafts. They were weighed, food cups and water bottles were filled and urine flasks were emptied at the same time each morning. Cages were cleaned at regular intervals. Rats were main-

tained in cages consisting of large galvanized iron cans equipped with no. 4 mesh wire screening as a bottom and with a removable top. These cages rested on 10 inch (25 cm.) glass funnels.

Diet.—Rats were given a standard diet, prepared in bulk, which contained 25 per cent free carbohydrate and 52 per cent total available carbohydrate.

Collection and Analysis of Urine.—Urine was collected in 125 cc. Erlenmeyer flasks containing about 1 cc. of toluene. No preservative was used when it was desired to apply fermentation tests to the urine.

Urinary dextrose was determined by titrating undiluted urine against 5 cc. of Benedict's quantitative solution. Urinary nitrogen was determined by a modification of the Folin-Wu [13] method, with the comparative colorimeter.

Collection and Analysis of Blood.—Blood was obtained for determination of dextrose by rapidly cutting off the tip of the animal's tail with a sharp razor blade. This procedure was usually painless, and blood was readily obtained with the aid of gentle massage of the cut tail. About 0.2 cc. of blood was collected for analysis, a small amount of potassium oxalate–sodium fluoride mixture serving as anticoagulant and preservative.

Dextrose was determined by a modification of the Folin ferricyanide reduction micromethod. The Evelyn photoelectric colorimeter was used; readings were converted into logarithmic equivalents and values for blood dextrose were read directly off a previously prepared and frequently checked curve.

Determination of Glycogen.—Tissues for determination of glycogen were obtained from the living animal anesthetized with pentobarbital sodium (5 mg. per hundred grams of body weight). As soon as anesthesia was complete the skin of the leg was incised and reflected back, so that the gastrocnemius muscle was exposed. The muscle was rapidly freed along its length and cut at the achilles tendon and proximally, a sample of about 1 Gm. being taken. This sample was immediately plunged into a 50 cc. tared centrifuge tube containing 2 cc. of 30 per cent potassium hydroxide solution. An abdominal incision was then made, a lobe of the liver clamped off and approximatealy 1 Gm. of hepatic tissue removed. This was quickly blotted on filter paper to remove excess blood and similarly dropped into a tared tube containing 30 per cent potassium hydroxide solution. Finally, 1 cc. of blood was drawn by cardiac puncture, for a check determination of dextrose.

Glycogen was precipitated with alcohol, hydrolyzed with hydrochloric acid and determined as dextrose by the Shaffer-Somogyi method. The blood filtrate was prepared by the Somogyi zinc precipitation method and similarly analyzed for dextrose.

Determination of Respiratory Quotients.—The open circuit Haldane apparatus was used for determining respiratory metabolism. The rat was placed in a Mason quart jar fitted with a large rubber stopper carrying an inlet and an outlet tube. Incoming air was purified by passing it successively through sulfuric acid and pumice, soda lime and anhydrous magnesium perchlorate.[14a] Thus it was freed of water and carbon dioxide. Emerging air was passed through two U tubes containing anhydrous magnesium perchlorate and two containing sodium hyroxide–asbestos absorbent [14b] and anhydrous magnesium perchlorate in that order. In this manner expired water and carbon dioxide were absorbed. The final tube was attached to a mercury manometer and a vacuum line, which drew the air through the system. The entire system (excluding the tubes used in purification of incoming air) was weighed before and after each trial. Carbon dioxide was determined directly, by the change in weight of the tubes containing sodium hydroxide–asbestos absorbent. The gain in weight of the system as a whole (rat + jar + U tubes) represented the oxygen consumed.

Liver Extracts.—Extracts "fraction A" and "fraction B" (the fractions used by H. B. Sokal in the treatment of patients with pernicious anemia) were specially prepared by the Wilson Laboratories, Chicago. The extract used most frequently in these experiments, which will be designated liver extract no. 1, was prepared frequently in small quantities by diluting with an equal volume of distilled water another extract specially prepared by the Wilson Laboratories. The diluted extract was a clear, deep brown, neutral solution, of specific gravity 1.14. Fifteen grams of fresh liver was represented by 1 Gm. of this extract; it contained 30 per cent of solid material and 2.6 per cent of ash. Its reducing power was equivalent to a 13.5 per cent solution of dextrose, and it contained 22 mg. of nitrogen per cubic centimeter. It was almost completely dialyzable. Other extracts, only briefly used, were Wilson's (1938), Lilly's (1939) plain and Abbott's (1940) concentrated liver extracts.

TABLE 1.—*Fermentation of Liver Extracts*

Extract	Dilution	Dextrose Equivalent	Yeast	Results
Fraction B	..	18.0%	Bakers'	Partial fermentation
L. E. 1	1-5	2.7%	Bakers'	No fermentation
L. E. 1	1.5	2.7%	Same sample bakers'	No fermentation
Dextrose	3%	3%		No fermentation
Dextrose	0.8%	0.8%		Slow fermentation
L. E. 1	1-5	2.7%	Brewers'	Slight fermentation
Lilly	1-10	0.6%	Brewers' and bakers'	Complete fermentation
L. E. 1	1-9	1.5%	Same sample mixed brewers' and bakers'	Slight fermentation
L. E. 1 plus 0.1 Gm. dextrose	1-9	2.0%		Fermentation equivalent to added dextrose
Dextrose	0.5%	0.5%	.....	Complete fermentation
L. E. 37	1-10	1.0%	.....	No fermentation
L. E. 37 plus dextrose	1-10	1.5%	Same sample bakers'	Partial fermentation
L. E. 38	1-10	1.0%	.....	No fermentation
L. E. 38 plus dextrose	1-10	1.5%	Same sample bakers'	Partial fermentation

These six commercial liver preparations, all at one time or another found to be efficacious in the treatment of pernicious anemia, were examined in this study. All were soluble in water, completely or with only small amounts of insoluble residue, forming a neutral, deeply colored solution. All were free of protein as judged by the heat precipitation, biuret, Millon and nitric acid tests. None contained a significant amount of other soluble material; all contained nitrogen.

A disturbing factor throughout the experiments has been the fact that four of the extracts tested, including all those capable of producing glycosuria consistently,

13. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38**:81, 1919.

14. (*a*) The preparation of anhydrous magnesium perchlorate used was "dehydrite." (*b*) The preparation of sodium hydroxide and asbestos was "ascarite."

gave strong reductions with Benedict's solution, equivalent to a dextrose concentration of 6 to 14 per cent. The extracts which reduced Benedict's solution behaved like equivalent solutions of dextrose toward the common sugar reagents. It was therefore attempted to identify the reducing material as dextrose. To this end, condensation with phenylhydrazine and fermentation tests were used. Various dilutions of three of the potent preparations were condensed with phenylhydrazine in the usual manner, dextrose solutions of equivalent reducing power being used as controls. In all cases crystalline precipitates were readily obtained which grossly resembled glucosazone and microscopically could not be distinguished from it. Fermentation tests with the liver extracts were rather unsatisfactory. Difficulty was experienced in obtaining potent samples of yeast and in achieving consistent results in attempts at fermentation of the extract. A complicating factor making the interpretation even more difficult was the presence of various preservatives in the extracts tested. The results of fermentation tests are listed in table 1. It will be seen that, although partial fermentation was obtained occasionally and complete fermentation was secured in 1 instance, the results on the whole cannot be regarded as satisfactory.

EXPERIMENTAL STUDIES

Production of Glycosuria by Parenteral Administration of Liver Extract and Assay of Several Preparations.—It was found possible to produce glycosuria consistently by subcutaneous or intraperitoneal injection of several liver extracts. In normal animals urinary excretion of dextrose of as much as 0.4 to 0.8 Gm. per day was obtained with large doses of extract. That the reduction noted was in reality due to dextrose was readily proved. Glucosazone was obtained by condensing samples of urine with phenylhydrazine; reduction of Benedict's solution was absent after fermentation of the urine with yeast.

The six liver extracts already mentioned were tested for ability to produce glycosuria. Three of these preparations are now in use in the treatment of pernicious anemia. The others, relatively cruder, were formerly found to be effective against that disease but have been replaced by more potent and purer preparations; these three, designated as "fraction A," "fraction B" and "liver extract no. 1," are samples prepared to resemble the extracts used by H. B. Sokal [12] in the work previously cited. The relative potency of these extracts was tested by administering various amounts of each parenterally to normal animals, under well controlled conditions. In some cases it was immediately apparent that an extract either had little ability to produce glycosuria or was potent enough to warrant further study; in other cases an extended period of observation was necessary to reach a conclusion.

Chart 1 presents a typical study of this sort. In this experiment, "fraction A" and "fraction B" were compared; three normal male guinea pigs weighing 250 to 300 Gm., on a diet of oats and lettuce, were used. Guinea pig S 1 was given an injection of "fraction B"; animal S 2 received an equal dose of "fraction A" under the same conditions; S 3, a control, was occasionally given an injection of dextrose solution. It will be noted that the control animal at one time excreted a significant amount of dextrose and that the experimental animals did not do so unless they were given liver extract. Administration of 0.2 cc. of the extract per hundred grams of body weight did not produce a glycosuric response. Administration of 0.4 cc. per hundred grams of either fraction A or fraction B evoked glycosuria; the response to fraction B was in every case greater than that to fraction A. Furthermore, after twenty-three days fraction A was no longer capable of eliciting glycosuria, whereas fraction B was as active as before. Doubling the dose of liver extract, to 0.8 cc. per hundred grams of body weight, resulted in a 69 per cent increase in urinary dextrose. Injections of dextrose solutions, alone or in conjunction with liver extract, had no effect on glycosuria. The possibility that individual variation played a role was ruled out by later giving animal S 2 an injection of fraction B, when a glycosuric response was again obtained in this animal.

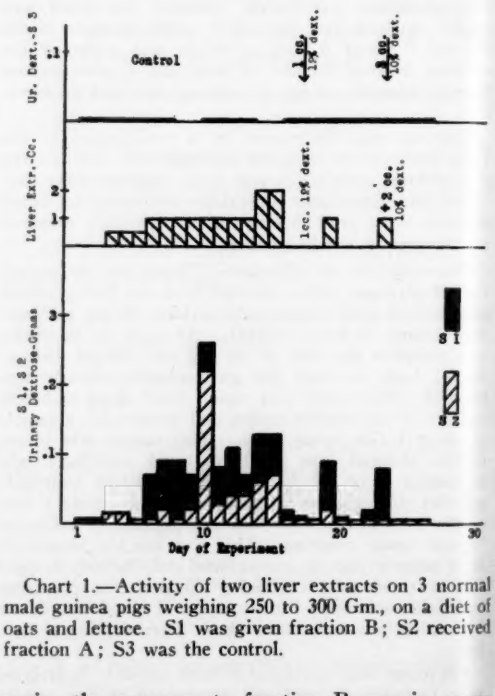

Chart 1.—Activity of two liver extracts on 3 normal male guinea pigs weighing 250 to 300 Gm., on a diet of oats and lettuce. S1 was given fraction B; S2 received fraction A; S3 was the control.

Table 2 summarizes the results of comparative studies of potency of the various extracts

The extracts were tested in some cases in the same animal at different times; in other cases in 2 animals of the same sex, age and weight, maintained under identical conditions. If the latter type of test was used, individual variation was ruled out by changing extracts at the conclusion of the experimental period, as first described. It will be seen that the most active preparations were fraction B and liver extract no. 1.

TABLE 2.—*Activity of Several Liver Extracts*

Extract	Description	Reduction Dextrose Equivalent	Experimental Animal	Glycosuria Produced
Wilson	Now manufactured for treatment of pernicious anemia	0	Normal guinea pig	1 plus
Fraction A	No longer used	10%	Normal guinea pig, normal mouse	2 plus
Fraction B	No longer used	12%	Normal guinea pig, normal mouse, normal and diabetic rat	4 plus
L. E. 1	No longer used	13.5%	Normal and diabetic rat	4 plus
Lilly	Now used in the treatment of pernicious anemia	6.0%	Normal rat	2 plus
Abbott	A "concentrated" liver extract, relatively pure, now used in the treatment of pernicious anemia	1.3%	Normal rat	0

Selection of Experimental Animals and Standardization of Procedure.—Guinea pigs, mice and rats were given injections of potent liver extract, and glycosuria was obtained in each species. Mice proved to be unsuitable, however, because only small amounts of urine were obtained and samples were usually grossly contaminated with feces. Both guinea pigs and rats were satisfactory, but the latter proved much more convenient as experimental animals because it was possible to house them in the departmental rat colony and feed them a standard diet of known composition. Accordingly, normal and depancreatized rats were the animals used in the subsequent experiments. The care and feeding of these animals and the collection of urine have already been described under "Methods."

It has been pointed out that fraction B and liver extract no. 1 were the two extracts found to be highly potent. These were very similar, and since liver extract no. 1 was somewhat the more active of the two and a larger quantity of it was available, it was decided to use that preparation in the remainder of the experiments. In the work to be reported in the rest of this paper, therefore, liver extract no. 1 is the extract used unless specific mention is made to the contrary.

Both subcutaneous and intraperitoneal injections were used. The former frequently caused a superficial ulcer at the site of an injection; furthermore, it was sometimes possible for a significant portion of the dose administered to be extruded after its injection. Intraperitoneal injections were more satisfactory on the whole; they had the disadvantage, however, of occasionally resulting in a fatal accident. Injections were usually made between 10 and 11 o'clock in the morning, under the same conditions from day to day.

Studies on Glycosuria Produced by Liver Extract.—Daily determinations of urinary dextrose were made on three types of experimental animals under the influence of active preparations of liver extract. The effects of various levels of dosage and of food intake were studied.

Chart 2 shows the dextrose excretion of a normal rat having free access to food and water, over a period of forty days. Several dose levels of "fraction B" were used, and on several occasions the animal was given an injection of dextrose solution. The graph illustrates a somewhat more uniform response than was usually obtained but is otherwise typical. It is seen that the animal excreted significant amounts of dextrose only when given injections of liver extract and that the amount excreted was proportional to the dose of the extract administered. The threshold dose was found to be 0.25 cc. per hundred grams of body weight; 130 mg. of dextrose was excreted at that level. With a

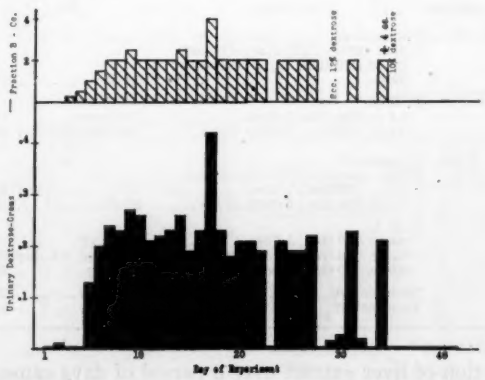

Chart 2.—Excretion of dextrose by a normal male rat weighing 400 Gm., on a standard diet, given injections of an active liver extract.

dose of 0.5 cc. per hundred grams, the urinary excretion rose to about 210 mg., and on one occasion, when 1 cc. per hundred grams was administered, 420 mg. of dextrose appeared in the urine. Injection of dextrose solution, alone or with liver extract, had no effect on urinary dextrose.

In other experiments with normal animals it was found that the excretion of dextrose occurred in the first six hours after administration of liver extract. Glycosuria was diminished but not abolished when liver extract was administered in the fasting state. Injection of liver extract in conjunction with feeding of dextrose solution by stomach tube caused a twofold to threefold increase in urinary dextrose; this interesting experiment will be discussed later. Feeding of dextrose alone caused no glycosuria.

Two rats with "latent diabetes" were available. These were partially depancreatized animals that manifested no glycosuria normally, but were susceptible to the "diabetogenic"-hormone of the anterior lobe of the pituitary gland. These animals behaved in general like normal rats toward one or two doses of liver extract, although they were somewhat more sensitive to its action, excreting 30 to 50 per cent more dextrose than normal rats receiving the same dose. It was noted, however, that administration of liver extract over a period of days caused a general increase in the glycosuria produced by a given dose; and in one animal a "diabetic" state was produced which persisted for two days after the last injection. These findings are reported in table 3.

TABLE 3.—*Effect of Liver Extract and Anterior Pituitary Extract on Two Partially Depancreatized Rats*

Animal	Injection	Urinary Dextrose, Mg.	
S 4	Foreperiod		0
	Saline anterior pituitary extract		
	0.5 cc./100 Gm. on 2 successive days	2d day	250
	L. E. 1		
	0.5 cc./100 Gm., 1 dose	Average	150
	0.5 cc./100 Gm., 5 daily doses	Average, 5th day	180
Y 128	Foreperiod		0
	Saline anterior pituitary extract		
	0.5 cc./100 Gm., 1 dose		210
	0.5 cc./100 Gm., 2 daily doses	2d day	550
	L. E. 1		
	0.5 cc./100 Gm., 1 dose	Average	160
	0.5 cc./100 Gm., 5 daily doses	Average, 5th day	230
	0.5 cc./100 Gm., 9 daily doses	9th day	690
	No injection	10th day	220
	No injection	11th day	100

Finally, the effect of liver extract on the dextrose excretion of a spontaneously diabetic animal was studied. Y-125, a depancreatized rat about 2 years old, was followed for two hundred and fifty days, receiving injections of liver extract at various intervals during that time. Chart 3 shows this animal's weight, food intake and urinary dextrose, and the ratio of urinary dextrose to total available dietary dextrose over this period, in average values for five day periods. It is seen that although the correspondence is not exact, in general the peak levels of urinary dextrose were attained during or shortly after administration of liver extract. The ratio of urinary dextrose to total available dietary dextrose (available dietary dextrose = carbohydrate plus 60 per cent of protein plus 10 per cent of fat), a better index of severity of the diabetic state, shows an even closer relation than the other factors to injections of liver extract. It is seen, however, that there is no correlation between the output of dextrose and the dose of liver extract; in fact, the greatest concentration of the diabetic state of this animal occurred at a time when it was receiving small doses of extract every other day.

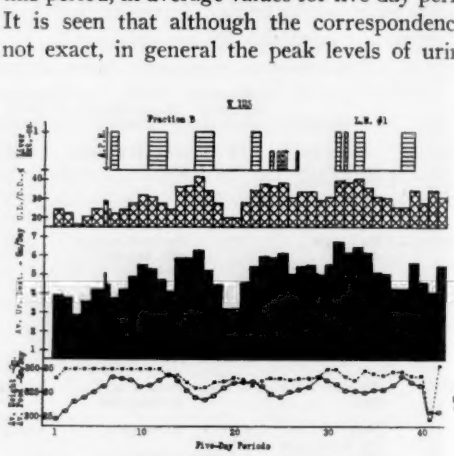

Chart 3.—Data on a diabetic rat given injections of active liver extracts. Weight, food intake, total dextrose excreted and dextrose excretion as percentage of available dietary dextrose were charted for 210 days in five day averages. U.D./D.D. % = $\frac{\text{Dextrose in urine}}{\text{Carbohydrate in diet 60\% of protein 10\% of fat}} \times 100$

A.P.E. indicates a single injection of saline extract of anterior pituitary, 0.5 cc. per hundred grams of body weight.

The intensity of the effect produced in this animal is best gaged by examining its previous record. During the month preceding the start of this experiment, its average daily output of dextrose was 4.02 Gm. Injection of a saline extract of the anterior lobe of the pituitary increased the excretion of dextrose from 4.3 to 5.1 Gm. The highest single day's output at any time previous to the injections of liver extract

was 5.8 Gm. In the course of the experiment, however, an output of 7.5 Gm. was attained on three occasions. It is not fair, of course, to compare the effect produced by continued administration of liver extract with that obtained by only one dose of anterior pituitary extract; it is obvious, however, that this animal's diabetic state was at least as readily accentuated by liver as by pituitary extract.

Effect of Liver Extract on Blood Sugar of Normal Animals.—It was found that intraperitoneal injection of liver extract caused a remarkable rise in blood sugar in fasting animals. Figure 4 contains two of the blood sugar curves

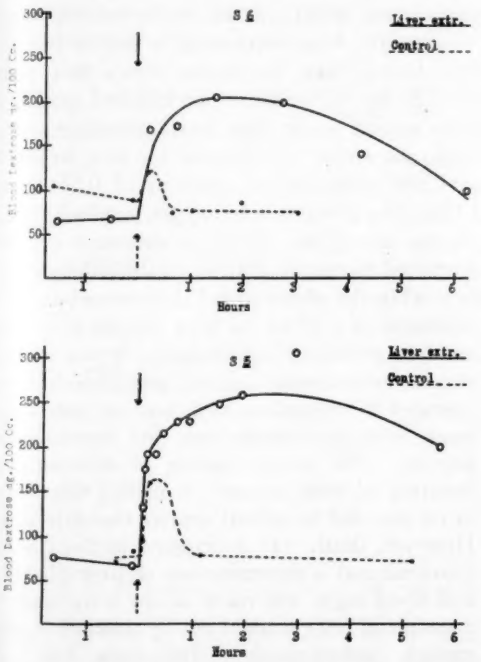

Chart 4.—Response of normal fasting rats to injections of liver extract. The control received 1.5 cc. of 14 per cent dextrose solution.

and is typical of the results obtained. S 5 and S 6 were normal female rats which were made to fast for thirty hours and were given injections of 0.67 cc. of liver extract no. 1 per hundred grams of body weight. Control curves represent the same rats given injections of 14 per cent dextrose solution under similar conditions. Table 4 presents the data obtained in the experiments on fasting normal rats given injections of liver extract. The blood sugar curves in chart 4 were compared with curves obtained after injection of 14 per cent dextrose solution. This procedure is valid only if the reducing power of the liver extract is due to dextrose, or to something readily metabolized to dextrose in the body. It is conceivable that the extract may contain a nondextrose reducing substance which is not rapidly disposed of by the organism and which would therefore persistently give false high values for blood sugar; this possibility was not rigorously excluded by the analysis of the extract.

TABLE 4.—*Effect of Liver Extract on Fasting Blood Sugar of Normal Rats* *

Time	Blood Sugar Values, Mg. per 100 Cc.	Average	No. of Observations
24-36 hour fasting.....	87, 70, 58, 75, 67, 105, 90, 85, 79, 67, 84, 62, 64, 40, 40	72	15
½ hour after injection of L. E. 1	200, 170, 156, 218, 167, 170, 130, 115	166	8
1 hour after injection...	173, 184, 230, 200	197	4
2 hours after injection..	182, 205, 258, 200	211	4
4 hours after injection..	240, 140	190	2

* Intraperitoneal injection, usually 0.67 cc. of liver extract no. 1 per hundred grams of body weight.

Accordingly the following experiment was performed:

A fasting animal was given an injection of liver extract. One hour later a lethal dose of insulin was administered and blood sugar values were obtained at intervals until the animal's death. If the high figures recorded in table 4 were due to a nondextrose reducing substance which persists in the rat's blood stream for several hours, the animal should have a relatively high blood "sugar" even though in insulin shock. If, on the other hand, these figures actually represented dextrose, the blood sugar reading should be extremely low at death. This experiment is recorded in table 5. It is seen that

TABLE 5.—*Effect of Insulin on the Rise in Fasting Blood Sugar Caused by Liver Extract*

Time, Hours	Injection	Comment	Blood Sugar, Mg. per 100 Cc.
0	.....	Fasted 12 hours	40
0	0.67 cc. L. E. 1/100 Gm.	.....	..
1	7 units insulin/100 Gm.	.....	131
1½	.....	.....	76
2	.....	Animal drowsy	51
2½	.....	Mild ataxia	41
3	.....	Tremors	10
4	1 Gm. dextrose.....	Comatose	0
5	.....	Roused slightly, died	10

rat S 5 succumbed with a blood sugar of 0, effectively excluding the presence of any reducing substance in the blood stream. Therefore, even if there is a noncarbohydrate reducing substance in the liver extract, it is not found in the blood of the experimental animal several hours after the injection.

In the course of some of the experiments to be reported an opportunity was afforded to examine the effect of intraperitoneal injection of liver extract on normal rats in the absorptive state. As might be expected, the blood sugar curves were similar to those obtained for fasting animals, but the levels of dextrose were considerably higher, reaching 425 and 350 mg. per hundred cubic centimeters in the two curves obtained. These figures are shown in table 6.

TABLE 6.—*Effect of Liver Extract on the Carbohydrate Stores of Normal Rats* *

Animal		Liver Glycogen, %	Muscle Glycogen, %	Blood Sugar, Mg. per 100 Cc.
S 9	Control, injection of dextrose every 4 hours for 24 hours	1.34	0.59	115
S 8	Injection of dextrose every 4 hours for 12 hours; then L. E. 1 every 4 hours for 12 hours	0.5	0.6	146
S 10	Same as S 8	0.52	0.36	70
S 11	Control, dextrose feeding plus injection of dextrose every 4 hours for 24 hours	6.0	1.34	130
S 12	Dextrose feeding plus injection of dextrose every	Died 17th hour		
S 13	4 hours for 12 hours; then dextrose feeding plus L. E.	Died 17th hour		
S 14	1 every 4 hours	0.33 Died 20th hour		14th hr. 320 16th hr. 360 20th hr. 425
S 16	Dextrose feeding; L. E. 1 at 4 hours; killed at 7 hours	2.06	0.54	230 ?
	Control (Cori-Cori [15])	(5.0)	(0.55)	(113)
S 17	L. E. 1; dextrose feeding at ½ hour; killed at 4½ hours	1.98	0.42	0-40 ½ hr. 113 1 hr. 139 2 hr. 270 3½ hr. 350 4½ hr. 350
	Control (Katzin [9])	(4.0)	(0.78)	(114)
	Control (Ferguson [20])	(3.1)	(0.68)	(147)

* All the animals were made to fast for twenty-four hours before the beginning of the experiment. Liver extract 1 and 14 per cent dextrose solution were given in doses of 0.67 cc. per hundred grams of body weight; the dextrose feeding was 720 mg. per hundred grams.

Effect of Liver Extract on Carbohydrate Stores of Normal Rats.—The effect of repeated injections of liver extract on muscle and liver glycogen of fasting rats was studied as follows:

Three rats were made to fast for twenty-four hours. They were then placed in metabolism cages, and urine was collected for twelve hours, during which time each rat received three injections of 0.67 cc. of 14 per cent dextrose solution per hundred grams of body weight. (This was done to obtain control values for studies of nitrogen balance, to be reported.) In the course of the next twelve hours, when second specimens of urine were being obtained, 2 animals received 3 injections of 0.67 cc. of liver extract no. 1 per hundred grams of body weight, while the third, a control, continued to receive the dextrose solution. At the end of the second twelve hours the animals were put to death, and values for blood sugar and for muscle and liver glycogen were determined.

The results are reported in table 6. It will be noted that there is a definite decrease in liver glycogen in the experimental animals; no conclusions can be drawn about muscle glycogen. The figure for liver glycogen of the control is higher than is ordinarily reported for fasting animals, but it must be remembered that this rat received dextrose every four hours.

It was attempted next to study the effect of repeated injections of liver extract on carbohydrate stores of fed animals, in the same manner. Accordingly, 4 rats were made to fast for twenty-four hours; then for twelve hours they were fed 720 mg. of dextrose per hundred grams of body weight every four hours (average maximum absorption of dextrose for this strain of rat) and given three injections of 0.67 cc. of 14 per cent dextrose solution per hundred grams. At the end of this period, 1 animal, a control, continued to receive dextrose orally and by injection, while the others were fed dextrose but given injections of 0.67 cc. of liver extract no. 1 per hundred grams of body weight. It was noticed that the experimental animals, which had hitherto tolerated the procedure well, became listless and weak, with poor muscle tone and depressed reactivity. The second feeding of dextrose and injection of liver extract intensified this state; in no case did an animal survive two injections. However, death was anticipated in the case of 1 animal and a determination of liver glycogen and blood sugar was made before it succumbed; a sample of muscle could not be obtained rapidly enough, unfortunately. The value for liver glycogen was 0.33 per cent, as against 6.0 per cent for the control animal.

The determinations of liver and muscle glycogen were made on animals that had fasted for twenty-four hours and received a single feeding of dextrose and one injection of liver extract. No controls were used, but one of the experiments was planned to duplicate the conditions under which Cori and Cori [15] studied the effect of epinephrine on carbohydrate stores in the postabsorptive state; the other simulated the experimental conditions under which Katzin [16]

15. Cori, C. F., and Cori, G. T.: The Mechanism of Epinephrine Action: II. The Influence of Epinephrine and Insulin on the Carbohydrate Metabolism of Rats in the Postabsorptive State, J. Biol. Chem. **79**:321, 1928.

16. Katzin, R.: The Role of the Adrenal Cortex in Carbohydrate Metabolism, Thesis, Yale University, 1940.

and Ferguson [17] obtained normal values in the strain of rat used in this study.

In the first experiment, a fasting rat was fed 720 mg. of dextrose per hundred grams. At the end of a four hour absorption period, the animal was given an injection of 0.67 cc. of liver extract no. 1 per hundred grams. Three hours later it was killed, and liver and muscle glycogen were determined. The value for muscle glycogen, 0.54 per cent, checked well with Cori and Cori's figures for control animals, but that for liver glycogen, 2.0 per cent, was distinctly lower than their value, of 5.0 per cent.

In the second experiment, a fasting rat was given an injection of 0.67 cc. of liver extract no. 1 per hundred grams of body weight and one half hour later fed 720 mg. of dextrose per hundred grams by stomach tube. At the end of four hours, the liver glycogen was 2.0 per cent and the muscle glycogen 0.42 per cent. In this case both muscle and liver glycogen are lower than Katzin's and Ferguson's figures for the normal rat under these conditions. It is noteworthy that the animal's blood sugar rose to 350 mg. per hundred cubic centimeters.

Effect of Liver Extract on Nitrogen Balance.—Studies of urinary nitrogen were performed to discover whether administration of liver extract caused any significant gluconeogenesis in addition to a mobilization of carbohydrate stores. Three normal rats were made to fast for twenty-four hours, in preparation for the first experimental period of twelve hours. During this period urine was collected (the bladders were expressed at the beginning and at the end of the twelve hours) and the animals were given injections of 0.67 cc. of 14 per cent dextrose solution per hundred grams of body weight every four hours. At the conclusion of this control study, a second collection of urine over twelve hours was made, and the 2 experimental animals were given an injection of 0.67 cc. of liver extract no. 1 per hundred grams every four hours, while the control continued to receive dextrose. Thus 1 animal served as a control over the nitrogen excretion of the two twelve hour periods, whereas each experimental animal served as its own control during the foreperiod of twelve hours. The findings are reported in table 7. It was not expected that accurate quantitative data would be obtained in this experiment, because of the relatively large amounts of nitrogen administered in the extract. However, it was felt that a significant gluconeogenesis should be revealed through the presence in the urine of excess nitrogen above that injected in the extract. This did not occur; in fact, a deficit of nitrogen was noted. This does not exclude the occurrence of gluconeogenesis, of course, since there is no evidence that the nitrogen in the extract is rapidly excreted.

It was hoped to throw further light on this question by repeating the experiment with dextrose-fed instead of fasting animals. In these circumstances gluconeogenesis would presumably be less likely to occur. Excess nitrogen found in the urine could then be taken as a rough measure of the excretion of nitrogen administered in the extract. Accordingly, an experi-

TABLE 7.—*Urinary Excretion of Nitrogen Under the Influence of Liver Extract (Preliminary Fast of Twenty-Four Hours)*

Animal	First 12 Hours	Urinary Nitrogen, Mg.	Urinary Nitrogen, Mg. per Hour per 100 Gm.	Second 12 Hours	Urinary Nitrogen, Mg.	Urinary Nitrogen, Mg. per Hour per 100 Gm.	Nitrogen Deficit,* Mg. per Hour per 100 Gm.
S 9	0.67 cc. 14 per cent dextrose solution per 100 Gm. every 4 hours	58	3.0	0.67 cc. 14 per cent dextrose solution per 100 Gm. every 4 hours	54	2.8	0.2
S 8	Same as S 9	80	3.4	0.67 cc. L. E. 1 per 100 Gm. every 4 hours	106	4.5	2.5
S 10	Same as S 9	48	2.8	Same as S 8	100	5.8	0.6
S 11	720 mg. dextrose solution per 100 Gm. every 4 hours	33	1.4	720 mg. dextrose per 100 Gm. plus 0.67 cc. 14% dextrose solution per 100 Gm. every 4 hours	43	1.8	Excess of 0.4
S 12	Same as S 11	39	1.6	720 mg. dextrose per 100 Gm. plus 0.67 cc. L. E. 1 per 100 Gm. every 4 hours; died 5th hour	28	2.7	1.8*
S 13	Same as S 11	62	2.7	Same as S 12, died 5th hour	24	2.8	2.8*
S 14	Same as S 11	91	3.5	Same as S 12; died at 8 hours	50	2.9	4.2

* Nitrogen deficit = nitrogen excretion first 12 hours plus nitrogen in extract injected minus nitrogen excretion second 12 hours. The second injection was neglected in the case of S 12 and S 13, because death occurred shortly afterward.

17. Ferguson, J. F., Jr.: The Role of the Thyroid Gland in Carbohydrate Metabolism, Thesis, Yale University, 1940.

ment which has already been described was undertaken. Four animals after a preliminary test of twenty-four hours were fed 720 mg. of dextrose per hundred grams of body weight every four hours and given an injection of 0.67 cc. of 14 per cent dextrose solution per hundred grams every four hours, for twelve hours. The control animal continued to receive dextrose for the next twelve hours, whereas the experimental animals were fed dextrose but received injections of liver extract. However, as has already been reported, all the experimental rats went rapidly downhill and died after the second injection. Under these circumstances it is highly doubtful whether urinary excretion of nitrogen would have any validity as a control for the preceding experiment. The figures obtained are reported in table 7, although no conclusions are drawn from the data.

TABLE 8.—*Effect of Liver Extract on the Respiratory Quotient of Normal and of Diabetic Rats*

Animal	R.Q., Fasting	R. Q., Fasting, Injections of Dextrose	R. Q., Fasting, Injections of Liver Extract	R. Q., Dextrose Feeding	R. Q., Dextrose Feeding, Injections of Liver Extract
Normal					
S 21	0.72	....	....	....	....
S 23	0.73	....	....	0.80	0.92
				0.87	0.91
	0.73	....	....	0.79	....
S 22	0.74	....	....	....	....
S 6	0.73	0.75	0.83	0.79	0.91
	0.74	....	....	0.87	....
S 5	0.73	0.75	0.79	0.87	....
				0.87	0.92
Average normal	0.73	0.75	0.81	0.85	0.92
Depancreatized					
Y 128	0.75	....	....	0.79	0.75
	0.74	....	....	0.72	....
Y 125	0.73	....	....	0.73	....
	0.70	....	....	0.72	0.74
Average, depancreatized	0.73	....	....	0.74	0.75

Dextrose feeding, 800 mg. per hundred grams of body weight of rat. Injections, 0.67 cc. of liver extract no. 1 or 14 per cent dextrose solution per hundred grams of body weight.

Effect of Liver Extract on Respiratory Quotients.—Respiratory quotients of rats under the influence of liver extract were determined in the fasting state and during absorption of dextrose. The Haldane open circuit apparatus was used, as described under "Methods." In all trials rats were made to fast for twenty-four to thirty hours before the beginning of the experiment. Determinations were started one hour after feedings of dextrose and one-half hour after injections of liver extract or 14 per cent dextrose solution; they lasted two hours. The results are reported in table 8. It is seen that liver extract was capable of raising the respiratory quotient of normal animals in either the fasting or the fed state but that there was no significant effect on the respiratory quotient of diabetic rats. The significance of these findings will be discussed later.

Some Properties of the Active Principle.—Active liver extract was administered orally to mice and rats. A glycosuric response was obtained in both groups of animals. An effective dose for administration by stomach tube in the rat was about three times that which would produce an equivalent response if given parenterally; no quantitative data were obtained on mice.

The effect of heat on the activity of the extract was next investigated. It was found that neither of the two potent fractions lost any significant proportion of their activity on being maintained at 100 C. for thirty minutes.

To study the effect of p_H on the activity of the liver extract, sulfuric acid and sodium hydroxide were added to two samples of liver extract no. 1, so that one sample contained 8 per cent acid by weight and the other 5 per cent alkali. The two solutions were then placed in a steam bath for fifteen minutes. After cooling, removal of the SO_4 ions and adjustment to neutrality, the two samples were compared in activity, the precautions outlined in the discussion of biologic assays being observed. It was found that the sample which had been incubated with acid was as active as before, while the one incubated with alkali had lost 40 per cent of its activity.

The active material was soluble in 60 per cent alcohol and was completely precipitated by 90 to 95 per cent alcohol. Its solubility in isopropyl alcohol was similar, but lower concentrations of this solvent served to cause precipitation. Centrifugation after such precipitations yielded a clear, deeply colored syrup of about one-fifth the volume of the original sample. None of the active principle was extracted from aqueous solution by butyl alcohol, acetone, chloroform or ether. The active material dialyzed somewhat slowly but completely through cellophane membranes.

Attempts at Fractionation of the Extract and Purification of the Active Principle.—Although it was not expected that isolation of the active factor could be achieved in the time available, several attempts were made at fractionation of the extract. This was done for two reasons. First of all, the extract used was highly hypertonic and obviously irritating when injected; removal of inactive material would undoubtedly

produce a less hypertonic and probably a less irritating extract. Also, if it were possible either to separate the active principle from the reducing substance in the extract or to prove that the two were identical, problems of experimental technic, of interpretation and of assay and eventual purification of the active material would be made much simpler.

It was soon found that dialysis was of no assistance. The extract used was almost completely dialyzable. Fractional dialysis was attended with no success in separating the various components of the extract, although theoretically this seeemed an attractive tool.

Neither acid nor alkali precipitated any portion of the extract. Saturation with ammonium sulfate had no effect. Addition of the common organic solvents resulted in complete solubility, complete insolubility or uniform precipitation of the extract as the percentage of water in the solution decreased. When precipitation with ethyl alcohol was 50 per cent complete, activity and reducing power were found in both fractions. Recourse was next to various "precipitants." Addition to trinitrophenol had no effect. Tannic acid produced a small amount of precipitate. Lead, mercury and silver were found capable of precipitating large amounts of the material in the extract. Of these, mercury was somewhat capricious in its action; silver nitrate seemed to precipitate almost all the solid in the extract, while basic lead acetate removed about half of the solid material.

It was impossible to test biologically all the fractions prepared. The lead separation was therefore selected for trial, as it seemed certain that whichever fraction proved active, a twofold purification would have been achieved. This proved to be the case. The lead precipitate was regenerated by dissolving in nitric acid, removing lead with sulfate, and adjusting to neutrality; it was found to be inactive and to contain only a trace of reducing material. The lead filtrate, on the other hand, retained practically all of the activity and reducing power of the original extract.

The phosphotungstic acid precipitation that Cohn and associates[18] found so useful in preparing pure preparations of the antianemic factor was now applied to the lead filtrate. This was acidified with sulfuric acid (to 5 per cent by weight), and 20 per cent phosphotungstic acid was added until precipitation was complete. The phosphotungstic precipitate was found divisible into alcohol-soluble, acetone-soluble and insoluble fractions. Material precipitated as phosphotungstate was regenerated with an excess of 20 per cent barium hydroxide, which removed phosphotungstate by precipitation as the barium salt. Injectable solutions of the various fractions obtained in this separation were prepared by precipitating toxic substances (phosphotungstate with barium; barium with sulfate) and adjusting to neutrality.

When the fractions obtained were tested biologically, in normal rats previously standardized with liver extract no. 1, it was found that a considerable loss of activity had occurred during the separation. Neither the regenerated phosphotungstate precipitate nor any of its fractions were active. The phosphotungstate filtrate was

TABLE 9.—*Fractionation of Active Liver Extract*

Fraction	Description	Reducing Power *	Activity
L. E. no. 1	Control, known activity.....	++++	++++
3 4 5 6 7	Samples taken at various stages in the dialysis of liver extract no. 1	+++ to ++++	+++ to -
16	L. E. no. 1 in 80% alcohol, precipitate.....	++++	++++
17	L. E. no. 1 in 80% alcohol, supernatant.....	++++	++++
19	Filtrate after treatment of L. E. no. 1 with basic lead acetate.....	++++	++++
23	Regenerated basic lead acetate precipitate.....	+	±
27	L. E. no. 19 treated with phosphotungstic acid, regenerated precipitate.....	+	0
31	L. E. no. 19 treated with phosphotungstic acid, filtrate	+++	++
40	L. E. no. 31 in 50% alcohol, filtrate.....	+++	++
42	L. E. no. 40 in 90% alcohol, precipitate.....	++	++++

* Correction is made for dilution undergone in the course of the fractionation.

active, but only weakly so. Most of the reducing material remained in the filtrate; there was some loss of this also.

The phosphotungstate filtrate was further purified by adding an equal volume of absolute alcohol and discarding the precipitate obtained, and then adding more alcohol until a 90 per cent alcohol mixture was obtained. On centrifugation, a small amount of clear golden liquid was obtained. This was dissolved in water and found to be active. Unfortunately, enough of this material was not available for quantitative estimation of reducing power. From the qualitative test, however, it seemed that, although there was some reduction, it was considerably less than usually obtained with this degree of activity.

Table 9 summarizes the positive results obtained in these attempts at fractionation of the

18. Cohn, E. J., and others: The Nature of the Material in Liver Effective in Pernicious Anemia, J. Biol. Chem. **77**:325, 1928.

extract. Under "description" are listed only the definitive steps of the fractionation. Fractions were of course freed of toxic material and reduced to neutral aqueous solution before injection. Correction is made for the effects of simple dilution.

Pathologic Studies.—All animals used in this study were subjected to autopsy, either after natural death or at the conclusion of an experiment. No constant pathologic change was seen.

Sections of thyroid, heart, lung, spleen, kidney, adrenal and testis were taken from representative animals. Except for occasional pneumonic infiltration, no deviation from the normal microscopic appearance of these tissues was noted.

COMMENT

Glycosuria, Hyperglycemia and Glycogenolysis.—It has been definitely established that injection of liver extract into normal rats causes glycosuria, hyperglycemia and a fall in the level of liver glycogen. The glycosuria is undoubtedly a reflection of the hyperglycemia; it is confined to the first few hours after the injection, when the blood dextrose is above the renal threshold; it is not excessive in amount.

A categorical explanation of the hyperglycemia is a little more difficult. Several factors may play a role in the production of this phenomenon. There is no doubt that the concomitant breakdown of liver glycogen accounts for an important share of the increased blood dextrose, especially in the postabsorptive state. This is not enough, however, for discharge of all the liver glycogen in a fasting rat would not produce enough dextrose to raise the concentration in the body fluids to the levels found. Two other intrinsic sources of dextrose are available to the rat; one extrinsic source is unwillingly supplied him. It can be shown that in the fasting rat maximal discharge of liver glycogen plus utilization of all the carbohydrate supplied in the liver extract is still not enough to account for the dextrose produced. Either gluconeogenesis or discharge of muscle glycogen must be postulated.

Carbohydrate balances have not been reported in this study because the differences in the muscle glycogen values obtained are not statistically significant. It is possible, however, by making an approximate balance sheet, to account for the dextrose mobilized by assuming a breakdown of muscle glycogen in addition to utilization of injected carbohydrate and mobilization of liver glycogen. This is entirely in accord with the experimental data, for some decrease in muscle glycogen was actually observed. It can be stated, then, that at least in some animals liver extract produces a breakdown of both muscle and liver glycogen.

Gluconeogenesis.—No evidence has been obtained that liver extract stimulates gluconeogenesis; all the experimental results can be explained without assuming this phenomenon. On the other hand, it has not been demonstrated that gluconeogenesis does not occur. Clarification of this issue must await at least partial purification of the liver extract and accumulation of further data on muscle glycogen.

Ability of the Tissues to Metabolize Dextrose.—It has been demonstrated that administration of liver extract causes a rise in the respiratory quotient of normal animals. This is prima facie evidence that the ability of the tissues to metabolize dextrose is not impaired. It is possible however, to obtain high respiratory quotients by another mechanism—through the production of an acidosis with blowing off of the carbon dioxide.

If the action of liver extract were through a shift in bicarbonate balance of the blood, either because of the liberation of lactic acid or as a result of some other factor, an effect should be observed in diabetic as well as in normal animals. This was not the case. On correction of the values obtained by subtracting the 0.01 increase in respiratory quotient noted in depancreatized rats, it is found that administration of liver extract caused a rise in respiratory quotient of 0.05 in fasting and of 0.06 in dextrose-fed normal animals. These increases are significant.

It is seen, therefore, that the respiratory quotients of rats unable to metabolize dextrose did not change, while the respiratory quotients of normal rats increased under administration of liver extract. It must, then, be concluded that liver extract does not affect (adversely at least) the ability of the tissues to metabolize dextrose. It is likely that the increased combustion of dextrose noted in the normal rats is simply an expression of the law of mass action, since dextrose levels in their body fluids were markedly increased.

Mechanism of Action.—The problem now remains of the mechanism through which the action of liver extract takes place. It has already been pointed out that there is no diminution of the ability of the tissues to metabolize carbohydrate and that the effects observed can be accounted for by a discharge of liver and muscle glycogen.

This may take place as a result of one or more of the following possibilities:

(*a*) The liver extract used may contain some toxic substance which affects in an unphysiologic manner the glycogen storehouse of the body. An example of this type of action is the disturbance of carbohydrate metabolism occasionally found in catarrhal jaundice.

(*b*) The liver extract may contain or stimulate the release of some substance known to affect carbohydrate metabolism "physiologically." Thus, many chemicals (e. g., morphine) produce hyperglycemia through stimulation of the adrenal medulla.

(*c*) The extract may contain or stimulate the release of some active substance not hitherto known to affect carbohydrate metabolism.

The first of these possibilities seems unlikely, for if a toxin were being frequently administered it is probable that the organism would react either by developing an antibody to the foreign material or by gradually succumbing to its influence and manifesting signs of progressive toxicity. Furthermore, damage to the tissues involved might be demonstrable microscopically. However, this is not the case. Several animals were given repeated injections of liver extract for periods up to two hundred days (one quarter of their life span) without showing any alteration in the response to the extract or any sign of ill health. Furthermore, no constant histologic change could be found.

The second possibility is a much more probable one. Most drugs producing hyperglycemia do so through a stimulation of the adrenal medulla. However, there are certain important differences between the action of liver extract and that of epinephrine. Cori and Cori, after a thorough study of the action of epinephrine on carbohydrate metabolism,[19] concluded that the effects of that substance are due to a decrease in the utilization of carbohydrate by the tissues and a marked breakdown of muscle glycogen to lactic acid. They found that, although liver glycogen diminishes in the first fifteen minutes after an injection of epinephrine, the rate of glycogenesis from lactic acid soon exceeds the rate of hepatic glycogenolysis, so that liver glycogen is normal at the end of one hour and considerably increased at the end of three or four hours. Liver extract, on the other hand, has been shown not to decrease utilization of dextrose by the tissues, to cause only a moderate decrease in muscle glycogen and to cause a striking decrease in liver glycogen three or four hours after injection. Furthermore, although Cori and Cori were able to produce a considerable hyperglycemia in fasting rabbits and in rats in the absorptive state, they failed to do so in fasting rats.

Two points may be raised in question of the complete validity of the arguments just stated. First, the subcutaneous injection of epinephrine in the Cori experiments may not be entirely comparable in its effects on glycogen stores to the constant stimulation of the adrenal medulla that may be exerted by liver extract. This may be the case, although it seems somewhat improbable. It is difficult to see how a difference in liberation of epinephrine could reverse an effect on the respiratory exchange. Secondly, the failure to obtain significant hyperglycemia in fasting rats given injections of epinephrine, as well as the increase in liver glycogen noted, may be related to the dose used rather than to any specific action of epinephrine. Again, this may be so, although it seems unlikely that a hormone which has a definite and well authenticated action on blood sugar and on carbohydrate stores should in these experiments be the mechanism of an entirely dissimliar effect. And while it is true that the rats treated in this study were not, strictly, fasting animals, it is difficult to see how the administration of 93 mg. of carbohydrate per hundred grams of body weight to an animal maintaining a respiratory quotient of 0.79 for several hours would explain a difference of 130 mg. per hundred cubic centimeters in blood sugar four hours later.

It must be concluded, therefore, that the action of liver extracts in these experiments is not similar to the action of epinephrine on carbohydrate metabolism as described in the literature. This conclusion is supported by the work of Buttner,[1] who obtained a hyperglycemic response on injection of liver extract into adrenalectomized animals.

Reports of substances other than epinephrine or drugs acting through the adrenals which mobilize glycogen stores and produce a striking hypergycemia have not been encountered in a brief survey of the literature. It is suggested, therefore, that the actions reported in this study

19. Cori, C. F., and Cori, G. T.: The Mechanism of Epinephrine Action: I. The Influence of Epinephrine on the Carbohydrate Metabolism of Fasting Rats, with a Note on New Formation on Carbohydrates, J. Biol. Chem. **79**:309, 1928; II. The Mechanism of Epinephrine Action: The Influence of Epinephrine and Insulin on the Carbohydrate Metabolism of Rats in the Postabsorptive State, ibid. **79**:321, 1928; III. The Influence of Epinephrine on the Utilization of Absorbed Glucose, ibid. **79**:343, 1928; IV. The Influence of Epinephrine on Lactic Acid Production and Blood Sugar Utilization, ibid. **84**:683, 1929; V. Changes in Liver Glycogen and Blood Lactic Acid After Injection of Epinephrine and Insulin, ibid. **86**:375, 1930.

are due to a substance not hitherto known to affect carbohydrate metabolism and acting specifically on the mechanisms of formation or breakdown of glycogen. It is hoped that future investigation will shed light on the physiologic processes involved.

Feeding of Dextrose and Injection of Liver Extract.—Of 3 animals fed dextrose by stomach tube and given an injection of liver extract and four hours later again fed dextrose and given another injection of the extract, all died, 2 during the fifth hour and 1 during the ninth. Animals given repeated injections of liver extract but not fed remained well. The rats that were fed dextrose and given injections of the extract seemed well shortly after the first injection; when next seen, at the time of the second injection, they were listless and flabby and not as reactive as usually. After the second feeding and injection they rapidly grew weaker and were later found dead.

Autopsy of these rats revealed no evidence of peritonitis or any other consequence of an error in technic. Unfortunately, histologic sections were not obtained. Explanation of these mortalities will not be attempted until further studies are undertaken, but it is suggested that they may represent a fatal intensification of the disturbance in carbohydrate equilibrium which was produced by liver extract, similar perhaps (by analogy only) to the effect obtained by administering a large amount of carbohydrate to a diabetic patient in delicate equilibrium.

The Liver Extract.—Some progress has been made in fractionation of the liver extract, and it is hoped that a much purer preparation will be available for future experiments. The relation between reducing power and biologic activity is not entirely clear; if the activity of fraction 42 is confirmed it should be easy to demonstrate that the reducing substance and the glycogenolytic principle are separate entities. The nature of the reducing substance has not been definitely established; the formation of glucosazone and the resistance to fermentation indicate it to be fructose or mannose. It is hard to understand why either of these hexoses should be found in such concentrations in liver extract. Of the two, fructose is more probable as a tissue constituent.

Future Investigation.—It is obvious that many of the problems discussed here will require further study. Experimentation will be continued along the following lines:

1. Purification of the glycogenolytic principle.
2. Effect of liver extract on carbohydrate stores, with obtaining of enough data to make it possible to draw up a carbohydrate balance sheet.
3. Effect of liver extract on the medulloadrenalectomized animal.
4. Effect of liver extract on the lactic acid content of the blood.

SUMMARY AND CONCLUSIONS

The existence in some liver extracts of a substance, not identical with the antianemic factor, capable of producing glycogenolysis, hyperglycemia and glycosuria in the normal rat has been demonstrated. It has been shown that this principle does not depress utilization of carbohydrate by the tissues and that its action is therefore not similar to that of epinephrine.

The glycogenolytic principle is water soluble, heat stable, and dialyzable through cellophane. It is not a protein. It is less active when given by mouth than when introduced parenterally. It is resistant to acid, although it loses some activity in alkali. A partial purification has been accomplished.

Progress in Internal Medicine

GASTROENTEROLOGY

A REVIEW OF THE LITERATURE FROM JULY 1943 TO JUNE 1944

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AND
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(Continued from page 278)

DUODENUM

Diverticula.—Warren and Emery [237] review the literature on duodenal diverticula and report a study of 103 cases. No typical symptomatology was found. Diverticula in the first portion of the duodenum are usually of the false type, pockets associated in most instances with duodenal ulcer; those in the second portion are usually a true herniation of the mucosa through the muscular wall, a fair number of these containing inclusions of pancreatic tissue. In 4 of the cases there was evidence of inflammation within the sac. In a fifth case the diverticulum was surrounded by a phlegmonous inflammation; the patient died of a purulent meningitis, hepatitis and endocarditis, but the pathologist was unable to determine the relationship between these infections and the diverticulum. Pearse discusses [238] the indications for operation and describes a technic for the removal of peri-Vaterian diverticula.

Obstruction.—Morlock and Gray [239] report the case of a girl aged 16, who had attacks of painless vomiting intermittently all her life until at operation there was found an internal herniation of the third portion of the duodenum through a small rent in the mesentery, with resultant obstruction and dilatation of the duodenum. Incomplete rotation of the ascending colon was also present. The patient was cured. McCarty and Present [240] describe a mesenteric pouch hernia resembling a paraduodenal hernia and discuss the anatomy and clinical features of both types. Acute duodenal obstruction due to calcified tuberculous retroperitoneal glands is reported.[241]

Cyst.—A case of enterogenous cyst of the duodenum is added to the 9 previously reported in the literature.[242]

Rupture.—Johnson [243] reports on retroperitoneal rupture of the duodenum resulting from blunt trauma with cure effected by operation and reviews 52 cases from the literature.

Cancer.—Howard,[244] in reviewing the literature of carcinoma of the duodenum, found 55 instances recorded in 117,433 autopsies, an incidence of 0.047 per cent. In 10,340 cases of carcinoma of the intestines the incidence of carcinoma of the small intestine was 1.47 per cent. Of 163 cases of carcinoma of the small intestine, involvement of the duodenum was found in 37 per cent, of the jejunum in 37 per cent and of the ileum in 28 per cent. In the duodenum carcinoma was found in the first portion in 34 per cent, in the second portion in 45 per cent, and in the third portion in 21 per cent. Carcinomas of the duodenum are generally conceded not to arise from preexisting ulcers, are seldom associated with polyposis, most commonly occur in a stenosing form and less frequently assume a polypoid structure. They metastasize late and then usually involve only adjacent tissues. Few symptoms are produced until the lumen of the duodenum becomes obstructed. In the first portion of the duodenum the symptoms simulate pyloric carcinoma; in the second portion they simulate carcinoma of the ampulla of Vater, with jaundice, fever and sepsis; in the third portion they are characterized by obstruction with bile in the gastric contents.

237. Warren, H. A., and Emery, E. S.: Duodenal Diverticula with Special Reference to Their Symptomatology, Gastroenterology **1**:1085-1099, 1943.

238. Pearse, H. E.: The Surgical Management of Duodenal Diverticula, Surgery **15**:705-712, 1944.

239. Morlock, C. G., and Gray, H. K.: Congenital Duodenal Obstruction, Ann. Surg. **118**:372-376, 1943.

240. McCarty, R. B., and Present, A. J.: A Mesenteric Pouch Hernia Simulating Paraduodenal Hernia, Surg., Gynec. & Obst. **78**:643-648, 1944.

241. Solis-Cohen, L.; Levine, S.; Skversky, N. J., and Zaslow, J.: Acute Complete Duodenal Obstruction, Am. J. Surg. **62**:290-292, 1943.

242. Orgias, R.: Enterogenous Cyst of the Duodenum, Brit. J. Surg. **31**:90-93, 1943.

243. Johnson, M. L.: Traumatic Retroperitoneal Rupture of the Duodenum: Presentation of a Case and Review of the Literature, Arch. Surg. **48**:372-380 (May) 1944.

244. Howard, J. W.: Carcinoma of the Duodenum, Am. J. M. Sc. **206**:735-746, 1943.

Brunschwig and Templeton,[245] in discussing the roentgenographic diagnosis of neoplasms of the periampullary region of the duodenum, emphasize irregularities in the duodenal outline, stiffness of the involved segment, changes in the mucosal markings and Frostber's "reversed 3" sign. Cohn[246] reports 2 cases of carcinoma of the duodenum in which the diagnosis was made preoperatively on the roentgenologic demonstration of an abnormal mucosal pattern; in both the onset was insidious and asymptomatic until obstructive symptoms appeared; the cancer developed above the ampulla, and hence there was no jaundice.

Grove and Rasmussen[247] report obstructive jaundice in a 59 year old man in whom a benign papilloma of ampulla of Vater the size of a walnut was found and removed at operation. Watson[248] describes a carcinoma of the ampulla removed successfully in a two stage resection. A case of infrapapillary adenocarcinoma of the duodenum is added to the 46 already in the literature.[249]

A parapyloric duodenal adenocarcinoma simulated duodenal ulcer preoperatively and remained cured five years following operation.[250] Child[251] reports a case of pancreaticoduodenal carcinoma with symptoms of one month's duration in which radical pancreaticoduodenectomy was performed in one stage. The patient remained relatively free of symptoms for fourteen months, at which time reexploration revealed massive recurrence of the tumor.

Sarcoma.—Bisgard and Cochran[252] describe primary lymphosarcoma successfully resected, although the patient died three months later. Williams[253] reports a case of leiomyosarcoma which perforated, resulting in peritonitis and death of a 30 year old woman thirty-six hours post partum. In McCullough's[254] case a leiomyosarcoma was complicated by ulceration, sinus formation and metastatic abscesses of the liver, lungs and heart.

245. Brunschwig, A., and Templeton, F. E.: Roentgenographic Diagnosis of Neoplasms of the Peri-Ampullary Region and Head of the Pancreas, Radiology **41**:438-443, 1943.

246. Cohn, I.: Carcinoma of the Duodenum, Ann. Surg. **119**:342-350, 1944.

247. Grove, L., and Rasmussen, E. A.: Benign Papilloma of the Ampulla of Vater, Am. J. Surg. **64**:141-143, 1944.

248. Watson, K.: Carcinoma of Ampulla of Vater: Successful Radical Resection, Brit. J. Surg. **31**:368-373, 1944.

249. Felsen, J., and Wolarsky, W.: Primary Infrapapillary Adenocarcinoma of the Duodenum, Arch. Path. **36**:428-431 (Oct.) 1943.

250. Burke, E.; Perkel, L. L., and Gnassi, A. M.: Duodenal Carcinoma, Am. J. Surg. **62**:267-271, 1943.

251. Child, C. G.: Carcinoma of Duodenum: One-Stage Radical Pancreatico-Duodenectomy Preserving the External Pancreatic Secretion, Ann. Surg. **118**: 838-842, 1943.

252. Bisgard, J. D., and Cochran, R. M.: Primary Sarcoma of Duodenum: Resection with Head of Pancreas by One-Stage Whipple Operation, Am. J. Surg. **61**:425-429, 1944.

253. Williams, M. H. L.: A Case of Perforating Leiomyosarcoma of the Duodenum Associated with Parturition, M. J. Australia **1**:585-586, 1944.

254. McCullough, K.: Leiomyosarcoma of the Duodenum, New York State J. Med. **44**:1248-1249, 1944.

SMALL INTESTINE

Physiology.—Miller,[255] in reviewing the investigations carried on in his clinic by means of small intestinal intubation, discusses intestinal absorption and the effects of drugs and of intestinal obstruction on the motility of the small bowel. Hamrick[256] describes a method for the introduction of the Miller-Abbott tube in which air is injected into the stomach through the tube to aid its passage into the duodenum. Gius and Racely[257] describe a simple, easily constructed, portable suction device dependent on the fact that a tire pump will provide suction when the plunger is reversed and designed primarily for use with the Miller-Abbott tube. It is effective, is simple and can be managed almost entirely by the patient himself.

Van Liere and co-workers,[258] in studying the effects of anoxia, found that the motility of the small intestine of dogs was unaffected by the partial pressures of oxygen between 80 and 43 mm. of mercury and that of mice with pressures above 94 mm. In the colon, anoxia produced a diminution in the contractions of both the longitudinal and the circular muscles. The influence of various agents affecting the autonomic nervous system was studied by giving a powdered charcoal mixture to matched pairs of unanesthetized dogs by stomach tube, using one animal as a control and its mate as the experimental subject and killing both after an appropriate interval, to determine the distance the charcoal mixture had

255. Miller, T. G.: Observations on the Human Digestive Tract by Intubation, Proc. Chicago Inst. Med. **15**:118-129, 1944.

256. Hamrick, W. H.: A Technic for Introducing the Miller-Abbott Tube, U. S. Nav. M. Bull. **41**:1737-1742, 1943.

257. Gius, J. A., and Racely, C. A.: A New Portable Suction Apparatus for Use with the Miller-Abbott Tube, Surgery **15**:574-578, 1944.

258. Van Liere, E. J.; Northup, D. W.; Stickney, J. C., and Emerson, G. A.: The Effect of Anoxia on Peristalsis of the Small and Large Intestine, Am. J. Physiol. **140**:119-123, 1943.

traversed the small intestine.[259] Ergotamine increased the motility of the small intestine 27 per cent and neostigmine 38 per cent.

Ingelfinger[260] has written a comprehensive review of the effect of drugs, endocrine substances and vitamins on intestinal motility. Huidobro, Montero and Cuevas[261] found that nitroglycerin does not affect intestinal movements but amyl nitrate and, to a lesser extent, theophylline ethylenediamine decrease movement and tonus. Pitressin and, to a lesser extent, neostigmine increase the spontaneous motility of the small intestine. Atropine sulfate and morphine decrease the motor activity, and the latter slightly increases the tonus.

Meyer and associates,[262] having reported in previous papers that the salivary, gastric and pancreatic secretions are diminished in the aged, proceeded to study intestinal absorption by means of the Althausen galactose absorption test. At the end of thirty minutes the group with an average age of 20.5 years absorbed approximately 50 per cent more galactose than the group with an average age of 76.6 years. After sixty minutes the values for the two groups were approximately the same; after ninety minutes the older group had the higher values, thus giving further evidence of the delay in absorption.

Kajdi and Davison[263] studied the specific gravity, surface tension, p_H, trypsin and amylase content and the rate of flow of the duodenal fluid in 9 infants with normal digestion, 1 suffering from sprue and 1 from acute diarrhea. The patient with acute diarrhea had a pronounced reduction of duodenal trypsin and amylase. In the others the surface tension of the duodenal contents was stable in spite of variations in the rate of flow and concentration of enzymes and bile acids. Walker,[264] in in vitro studies, found that caffeine had no effect on the activity of the digestive enzymes: ptyalin, pancreatic amylase, pepsin, trypsin and pancreatic lipase. Coffee extract did not affect the digestion of casein by pepsin or trypsin; it increased the rate of digestion of starch by salivary and pancreatic amyalse and retarded the digestion of olive oil by pancreatic lipase.

Fink and Nasset[265] have provided a method for the bioassay of enterocrinin, the hormone which excites the secretory glands of the intestine, the principal feature of the method being that it gives accurate and reproducible results quickly by precise treatment of the time factor. A statistical analysis of data on 9 dogs shows a closer correlation between dose and response when the dose of enterocrinin is given without reference to the weight of the dog than when it is given on a per kilogram of weight basis. Secretin, likewise, was found to give more uniform responses in different dogs if the same dose was given, regardless of body weight, whereas the vasodilatins present in intestinal extracts are best expressed on a per kilogram basis. Fink[266] describes a procedure for obtaining highly potent preparations of enterocrinin. The potency has been increased to over three hundred times that of the crude extract.

Forster, Helm and Ingelfinger[267] conducted experiments in order to determine whether or not action potentials could be elicited from the intact human intestine and, if so, whether these potentials could be correlated with mechanical evidence of contraction and with the electrical potentials of smooth muscle as determined in animal experiments. In 1 patient with an ileostomy the contractions were observed directly and in 4 patients indirectly with use of a Miller-Abbott tube. The electrical potentials correlated with the mechanical evidence of contraction and in all essential respects were similar to the activity of isolated preparations of smooth muscle.

Radiosodium was employed as a tracer in measuring rates of sodium ion movement into and out of the gut at several levels in chronic surgically prepared segments of dog intestine.[268]

259. Van Liere, E. J.; Northup, D. W., and Stickney, J. C.: The Influence of Agents Affecting the Autonomic Nervous System on the Motility of the Small Intestine, Am. J. Physiol. **141**:462-465, 1944.

260. Ingelfinger, F. J.: The Modification of Intestinal Motility by Drugs, New England J. Med. **229**: 114-122, 1943.

261. Huidobro, F.; Montero, E., and Cuevas, F.: The Effect of Drugs on the Motility of the Jejunoileum in Normal Man, Surg., Gynec. & Obst. **78**:471-476, 1944.

262. Meyer, J.; Sorter, H.; Oliver, J., and Necheles, H.: Studies in Old Age: VII. Intestinal Absorption in Old Age, Gastroenterology **1**:876-881, 1943.

263. Kajdi, L., and Davison, W.: Chemical and Enzymic Studies of the Duodenal Contents of Infants, J. Pediat. **23**:204-214, 1943.

264. Walker, F.: The Effect of Caffein in Coffee Extract on the Activity of the Digestive Enzymes, Am. J. Physiol. **139**:343-346, 1943.

265. Fink, R. M., and Nasset, E. S.: The Physiological Response to Enterocrinin Considered Quantitatively, Am. J. Physiol. **139**:626-632, 1943.

266. Fink, R. M.: The Fractionation of Enterocrinin Preparations, Am. J. Physiol. **139**:633-637, 1943.

267. Forster, F. M.; Helm, J. D., Jr., and Ingelfinger, F. J.: The Electric Potentials of the Human Small Intestine, Am. J. Physiol. **139**:433-437, 1943.

268. Visscher, M. B.; Varco, R. H.; Carr, C. W.; Dean, R. B., and Erickson, D.: Sodium Ion Movement Between the Intestinal Lumen and the Blood, Am. J. Physiol. **141**:488-505, 1944.

Sodium ions move in both directions across the intestinal epithelium at measurable rates under all conditions studied. There is in general a descending aboral gradient in movement of sodium from blood to intestine, the movement being least in the colon. The same holds for movement in the intestine to blood direction, except that there is probably little difference between ileum and colon. The rates of movement both out of and into the gut are both positively correlated with the sodium ion concentration in the small intestine. The colon possesses a more efficient absorbing mechanism. The observations stress the importance of dynamic factors in determining the apparent permeability of living membranes.

Smith and Bergmenn [269] describe the preparation of l-leucine-aminoexopeptidase from swine intestinal mucosa together with evidence of the presence in swine mucosa of enzymes that hydrolyze various glycines.

Further information regarding the amount of small intestine required for the preservation of life is provided by 2 cases described by Prioleau,[270] In the first the patient survived the resection of 260 cm. of small intestine and 31 cm. of sigmoid colon followed by two subsequent major operations to reestablish the continuity of the bowel. In the second case the patient survived the removal of 354 cm. of small intestine and 40 cm. of sigmoid. The first patient, from whom approximately 40 per cent of the small bowel was removed, remained well; in the second, who lost an estimated 53 per cent of his small intestine, diarrhea, emaciation and edema developed and he died in four months. These observations are thus in accord with the view that the upper limit of tolerated resection is 50 per cent.

Roentgenology.—Schatzki [271] points out that in all parts of the gastrointestinal tract except the small intestine the examination is performed by observing roentgenoscopically the actual filling of the organ in question and then by studying the partially or completely filled organ. In the small intestine this may be accomplished by introducing barium sulfate solution through a duodenal tube. The barium reaches the cecum in an average time of fifteen minutes. The advantages of the method are: The small intestine is seen filled in its entirety; the actual filling of the small intestinal loops can be observed roentgenoscopically; dehydration of the barium mixture, as in the usual peroral examination of the small intestine, resulting in a lumpy incomplete filling of the ileum, does not occur; an adequate examination of the ileocecal valve and cecum can be made when a rectal enema is contraindicated; the time required for the examination is shorter than with the conventional method. The disadvantages of the method are: There are annoyances and difficulties associated with the introduction of the duodenal tube; the purpose of the examination is defeated if the filling of the small intestine takes place very slowly, due usually to reflux into the stomach; functional changes in the small intestine are best studied by the conventional examination. Cases in which the method is contraindicated consist of those cases in which a routine gastrointestinal examination is contraindicated, cases of suspected gangrene of the bowel and cases of active duodenal ulcer.

In 5 dogs hypoproteinemia and edema had no significant effect on the rate of intestinal absorption of galactose and aminoacetic acid.[272] Roentgenologic observation after the administration of barium sulfate showed in some of the roentgenograms of the small intestine during edema a moderate clumping of the barium and segmentation.

Anomalies.—Grove [273] reports duplication of the terminal ilium associated with two congenital diverticula. Glover and Garvin [274] report intestinal obstruction in a newborn infant in whom three anomalies were found at operation. Adhesive bands constricting the duodenum and a loop of jejunum passing through a hole in the mesentery together caused the obstruction and were associated with a third anomaly, partial volvulus of the nonrotated cecum. Recovery followed relief of the obstruction. Dolton [275] reports intestinal obstruction due to herniation through a mesenteric defect. Stock and Can-

269. Smith, E. L., and Bergmenn, M.: The Peptidases of Intestinal Mucosa, J. Biol. Chem. **153**:627-651, 1943.

270. Prioleau, W. H.: Massive Resection of the Small Intestine: Report of Two Cases, Ann. Surg. **119**:372-376, 1944.

271. Schatzki, R.: Small Intestinal Enema, Am. J. Roentgenol. **50**:743-751, 1943.

272. Beams, A. J.; Free, A. H., and Leonards, J. R.: Experimental Hypoproteinemia and Edema: Studies of Intestinal Absorption and Intestinal Roentgenologic Characteristics, Arch. Int. Med. **73**:397-402 (May) 1944.

273. Grove, E. W.: Duplication of the Terminal Ileum: Report of a Case Associated with Two Congenital Diverticula, South. M. J. **36**:735-736, 1943.

274. Glover, D. M., and Garvin, J. A.: Multiple Jejunal Congenital Anomalies with Intestinal Obstruction, Ohio State M. J. **39**:734-735, 1943.

275. Dolton, E. G.: Mesenteric Defects, Brit. J. Surg. **31**:275-277, 1944.

non [276] report on a 3 day old child with symptoms of intestinal obstruction found at autopsy to have a blind end of the small bowel 3 inches (7.5 cm.) beyond the duodenojejunal flexure.

Volvulus.—Barbosa [277] reports on volvulus of the entire small intestine treated by detorsion, with recovery.

Intussusception.—Good [278] discusses the diagnosis of enteroenteric intussusception roentgenologically after administration of barium sulfate by mouth. The characteristic observations are: gradual narrowing of the lumen of the bowel as it approaches the intussusception; pronounced narrowing of the lumen through the intussusception; retrograde filling of the space between the intussusceptum and the intussuscepiens, with the formation of a characteristic "concentric ring" or "spiral sheath" appearance, and a palpable mass coincident with a filling defect in the barium column. Though enteroenteric intussusception is usually secondary to some other lesion of the bowel, the latter is seldom identifiable by the examination. Good considers the administration of barium by mouth to be safe if a barium sulfate enema has excluded obstruction of the colon.

Gibbs and Sutton [279] review 92 cases of intussusception in infants and children observed over sixteen years. Scott [280] presents a case of jejunal intussusception, thought to be the second in the literature. The patient was a 6 day old infant, with vomiting and passage of blood by rectum. Clark [281] reports on an irreducible double intussusception due to an invaginated Meckel diverticulum complicated by volvulus of the affected portion of bowel. Leiter [282] reports ileoileal intussusception due to a Meckel diverticulum in a 14 year old boy. Recovery followed resection of the intussusception with ileotransverse colostomy.

Stenosis and Adhesions.—A 61 year old man with episodes of abdominal pain accompanied with nausea and vomiting was found at operation to have a strangulated bowel.[283] It was freed of adhesions, but the attacks of abdominal pain continued. At the second operation the stenosis was found to be caused by cicatricial tissue involving 8 cm. of the bowel. Henry [284] records a 30.6 per cent mortality in 219 cases of nonmalignant intestinal obstruction. In 77 cases simple release of adhesions resulted in a 15.5 per cent mortality. In 29 cases enterostomy for decompression was carried out, with a 41.4 per cent mortality. In 35 cases resection was necessary, the mortality being 57 per cent.

Bezoar.—A trichobezoar in the terminal portion of the ileum resulted in volvulus, infarction of the bowel, peritonitis and death in a 13 year old girl.[285]

Rupture.—Ficarra [286] reports 16 interesting cases of nonpenetrating abdominal injuries with traumatic perforation of the small intestine.

Aneurysm.—Hiller and Johnson [287] review the literature of rupture of abdominal aneurysms in the intestinal tract and report on a 76 year old white man who complained for six weeks of constant epigastric pain, anorexia, intermittent nausea and abdominal distention. The guaiac test of the stools gave positive results on several examinations; the result of a roentgenologic examination of the gastrointestinal tract was reported negative. The patient suddenly became semicomatose, passed large amounts of blood by rectum and died. An arteriosclerotic aneurysm of the abdominal aorta perforating into the jejunum was present at autopsy.

Gallstone Ileus.—Rankin and Eger [288] report 3 cases of intestinal obstruction due to gallstones. In 2 the obstruction was high in the jejunum and in the third at the ileocecal junction. Nitkin

276. Stock, F. E., and Cannon, D. A.: Atresia of the Small Intestine, Brit. J. Surg. **31**:96-97, 1943.

277. de Castro Barbosa, J.: Volvulus of the Entire Small Intestine and Its Mesentery, Am. J. Surg. **64**: 400-404, 1944.

278. Good, A. C.: Enteroenteric Intussusception, Radiology **42**:122-127, 1944.

279. Gibbs, E. W., and Sutton, P. W.: Intussusception—Ninety-Two Cases in Infancy and Childhood, Surgery **14**:708-718, 1943.

280. Scott, E. P.: Jejunal Obstruction: Report of a Case in a Six-Day-Old Infant, J. Pediat. **23**:565-567, 1943.

281. Clark, C. W.: Irreducible Double Intussusception Due to Meckel's Diverticulum Complicated by Volvulus, Brit. J. Surg. **31**:301-303, 1944.

282. Leiter, H. E.: Intussusception Due to Meckel's Diverticulum: Recovery Following Resection and Ileo-Transverse Colostomy, J. Mt. Sinai Hosp. **10**:789-791, 1944.

283. Tanner, N. C., and Bratton, A. B.: A Case of Intestinal Stenosis Following Strangulation with a Pathological Description, Brit. J. Surg. **31**:88-90, 1943.

284. Henry M. J.: Non-Malignant Intestinal Obstruction, South. M. J. **37**:69-72, 1944.

285. Forbes, R. P.: Bezoar Causing Intestinal Obstruction, J. Pediat. **24**:574-576, 1944.

286. Ficarra, B. J.: Traumatic Perforations of the Small Intestine Due to Non-Penetrating Abdominal Injuries, Surgery **15**:465-475, 1944.

287. Hiller, G. I., and Johnson, R. M.: Abdominal Aortic Aneurysm: Rupture into the Jejunum Preceded by Ocult Blood in the Stool, Am. J. M. Sc. **207**:600-606, 1944.

288. Rankin, L. M., and Eger, S. A.: Intestinal Obstruction Due to Gallstone, Am. J. Surg. **61**:445-448, 1944.

and Lesser [289] describe intestinal obstruction due to gallstone with preoperative roentgen visualization of the biliary radicles. Of 36 such roentgenologically studied cases previously reported in the literature visualization of the biliary tract was observed preoperatively in 22. The following signs of gallstone ileus are recognized: air or contrast medium in the biliary tract; complete or partial intestinal obstruction as noted by distended loops of bowel, with visualization of the stone by a plain film or by the ingestion of a barium sulfate meal permitting the outlining of a radiolucent calculus; change in position of a previously observed stone. Utilization of intestinal intubation preoperatively contributes materially to the management in such cases by decompression of the bowel and by permitting the administration and subsequent removal of barium for the identification of the obstruction.

A 74 year old woman with a history of biliary colic after five days in the hospital for biliary colic had sudden acute pain in the lower part of the abdomen followed by symptoms of acute intestinal obstruction.[290] The Miller-Abbott tube was passed; the general improvement was so striking that operation was not performed. Roentgenologic examination showed a stone in the colon and later two big stones were passed in the stools. Gastroduodenal roentgenograms disclosed a cholecystoduodenal fistula.

Diverticula.—Benson, Dixon and Waugh [291] present results of an interesting study of 122 cases of so-called nonmeckelian diverticula, in 100 occurring in the jejunum, in 17 in the ileum and in 5 throughout the intestine. Symptoms developed only when complications occurred, as they did in 13 cases, chronic intestinal obstruction being the most frequent complication. The first case of carcinoma of a jejunal diverticulum is reported. D'Abreu [292] reports the unusual case of a 37 year old man with epigastric pain after meals, relieved by alkalis and aggravated by meat, associated with colic-like abdominal pain and alternating diarrhea and constipation. At operation a huge diverticulum of the small bowel was found at the junction of the jejunum and the ileum.

A Meckel diverticulum 18 inches (46 cm.) from the ileocecal valve was visualized by a barium sulfate enema.[293] Calcified concretions are described in a Meckel diverticulum.[294] The tip of the diverticulum was attached to the tip of the appendix, forming a band that caused mechanical obstruction. Rudder [295] reports acute diverticulitis of the jejunum, with resection and uneventful recovery.

Treatment of Intestinal Obstruction.—Grimson and Hodge [296] discuss the use of prolonged intubation suction technic in the treatment of intestinal obstruction produced by postoperative or inflammatory adhesions and present 7 cases illustrating the success of this method. However, patients with strangulation or recurring attacks of obstruction in spite of conservative treatment require exploration. Bennett [297] also discusses the diagnosis and treatment.

Sprue and Related Disorders.—Crohn [298] reviews the recent advances made in the study of the diseases of the small bowel, including sprue, the deficiency states, ileojejunitis and regional enteritis. Sprue is interpreted by Wilder [299] as one type of jejunoileal functional insufficiency with diminution in absorption of the products of digestion as well as of minerals and vitamins. Since fats are less readily absorbed than proteins or carbohydrates in normal persons, it is readily understandable that inadequate absorption of fat should be responsible for many of the symptoms. The excessive amounts of split fat and soaps in the stools are characteristic. In the active stages of the disease fat in the form of fatty acids and soap may constitute 50 per cent or more of the dried weight of the stool. Lack of absorption of fat causes serious deficiencies of all the fat-soluble vitamins, A, D and K, and excessive loss

289. Nitkin, R. L., and Lesser, A.: Intestinal Obstruction Due to Gallstone, Ann. Surg. **118**:101-106, 1943.

290. Owens, F. M., Jr.: Gallstone Ileus: Successful Treatment with the Miller-Abbott Tube, Gastroenterology **1**:938-941, 1943.

291. Benson, R. E.; Dixon, C. F., and Waugh, J. M.: Nonmeckelian Diverticula of the Jejunum and Ileum, Ann. Surg. **118**:377-393, 1943.

292. d'Abreu, F.: An Unusual Case of Diverticulum of the Small Intestine, Brit. J. Surg. **31**:408-409, 1944.

293. Poppel, M. H.: The Roentgen Demonstration of Meckel's Diverticulum, Am. J. Roentgenol. **51**:205-206, 1944.

294. Gile, J. F., and MacCarty, W. C.: Calcified Concretions Within a Meckel's Diverticulum, Radiology **41**:491-494, 1943.

295. Rudder, F. F.: Acute Diverticulitis of the Jejunum—Case Report, Surgery **14**:921-923, 1943.

296. Grimson, K. S., and Hodge, G. B.: Prolonged Intubation Suction and Deferred or Delayed Surgery in the Treatment of Multiple Adhesive Obstructions of the Small Intestine, Surg., Gynec. & Obst. **78**:316-326, 1944.

297. Bennett, L. C.: Diagnosis and Treatment of Obstruction of the Small Intestine, Am. J. Surg. **62**: 59-64, 1943.

298. Crohn, B. B.: Benign Diseases of Small Intestine, Gastroenterology **2**:385-394, 1944.

299. Wilder, R. M., Jr.: The Nontropical Sprue Syndrome: Report of Four Cases and of a Case in Which Intestinal Insufficiency Was Corrected by Operation, Proc. Staff Meet., Mayo Clin. **19**:297-302, 1944.

of calcium in the stools. Delayed absorption of dextrose causes a flattened curve in the dextrose tolerance test. Likewise, vitamin B complex, vitamin C and iron are poorly absorbed. Four typical cases are described, with prompt response to a low fat diet and large doses of minerals and vitamins. A fifth and very instructive case is one in which a gastroenterostomy had inadvertently been done between the stomach and lower part of the ileum, with resultant fatty diarrhea (62 per cent of the dry weight) and low level of plasma vitamin C. Prompt recovery occurred after the gastroenteric stoma was taken down. Adlersberg and Sobotka [300] have also called attention to the inability of the small intestine to absorb fat and the fat-soluble vitamin A during an attack of sprue whereas during a remission after adequate treatment there is fairly satisfactory absorption of both. The fat tolerance and vitamin A absorption tests are described in four groups of patients: control, with active sprue, with inactive sprue and with jejunoileitis.

McIntosh,[301] in discussing the disorders of the digestive system leading to vitamin deficiency, states that the failure of absorption of fat-soluble vitamins A and K, and to a less extent vitamin D, occurs in diseases with jaundice and acholic stools, in fibrosing pancreatitis and in celiac disease. Fibrosing pancreatitis, often mistaken for celiac disease, is differentiated from it by the demonstration of an absence of the normal pancreatic enzyme from the duodenal contents under controlled conditions. Hence, if the examination of the stool for fat discloses a significant degree of impaired fat absorption, one must be prepared to go ahead with procedures leading to a more certain appraisal of pancreatic performance.

In an effort to classify recurrent and chronic diarrhea of infancy and childhood, the type of metabolic diarrhea known as the "celiac syndrome" has been divided by the Lapin [302] into five subtypes, namely: cystic fibrosis of the pancreas, intolerance to starch, infantile steatorrhea, celiac disease and allergic celiac disease. The evidence supporting this differentiation is not entirely satisfactory to us, but the emphasis on quantitative chemical analyses of the stool for fat and starch is amply justified.

300. Adlersberg, D., and Sobotka, H.: Fat and Vitamin A Absorption in Sprue and Jejunoileitis, Gastroenterology **1**:357-365, 1943.

301. McIntosh, R.: Disorders of the Digestive System Leading to Vitamin Deficiency States in Infants and Children, Bull. New York Acad. Med. **20**:25-33, 1944.

302. Lapin, J. H.: Recurrent and Chronic Diarrhea in Infancy and Childhood, Am. J. Dis. Child. **67**:139-143 (Feb.) 1944.

Fritzsche [303] reports on lymphosarcomatosis of the small intestine and of the mesenteric lymph nodes in which the clinical picture was that of endemic sprue with chronic fatty diarrhea, swelling of the abdomen, emaciation, mild anemia of the secondary type and roentgenologic evidence of a severe jejunitis, ileitis and colitis. The disturbance in the absorption of fat was apparently explained by the extensive infiltration in the small intestine, particularly the jejunum and upper portion of the ileum and in the mesenteric lymph nodes.

Morlock and Rosenberg [304] report the unusual association of nontropical sprue and severe tophaceous gout.

Forbes and Atkinson [305] describe a relatively simple titration method for the determination of neutral fat, including sterols, and of the fatty acids, including both fatty acids and soaps, in the feces. Volk and Popper [306] urge use of the fluorescent microscope for the demonstration of fat droplets in urine and feces as a better and simpler method than staining with sudan III.

Regional Enteritis.—The perplexing problem of regional enteritis is well discussed in a recent editorial.[307] Pathologically the distinguishing features are: 1. Hypertrophy and thickening of the wall of the bowel, usually confined to some one localized stretch of the mesenteric small intestine, most frequently the terminal ileum, and varying in extent from a few inches to several feet. A bizarre feature of the disease is its occasional tendency to attack more than one segment of the bowel, leaving the intervening segments intact. The process may involve the colon down to the sigmoid. 2. Resultant narrowing of the lumen of the bowel. 3. Hyperplasia of the mucosa, often with ulceration. 4. Perforation, with local or general peritoneal involvement and establishment of internal or external fistulas. This train of events is usually preceded by hyperplasia of lymphatic tissue and an obstructive

303. Fritzsche, R.: Syndrom von symptomatischer Sprue bei Lymphosarkomatose des Dünndarms und der mesenterialen Lymphdrüsen, Schweiz. med. Wchnschr. **73**:442, 1943.

304. Morlock, C. G., and Rosenberg, E. F.: The Coexistence of Tophaceous Gout and Non-Tropical Sprue: Report of a Case, Ann. Int. Med. **20**:981-989, 1944.

305. Forbes, J. C., and Atkinson, T. T.: The Separate Determination of the Fatty Acid Fraction and of the Neutral Fat Plus Sterol Fraction in Feces, J. Lab. & Clin. Med. **28**:1507-1510, 1943.

306. Volk, B. W., and Popper, H.: Microscopic Demonstration of Fat in Urine and Stool by Means of Fluorescence Microscopy, Am. J. Clin. Path. **14**: 234-238, 1944.

307. Regional Ileitis, editorial, J. A. M. A. **126**:499 (Oct. 21) 1944.

lymphedema. Microscopically the appearance is highly mimetic of tuberculosis; but all attempts to demonstrate tubercle bacilli or any other causative organism, including the virus of lymphogranuloma venereum, have failed. The counterpart of the disease has not been observed in animals. The diagnosis rests on symptoms so complex and varied as to create an almost invariable hazard. Crohn's original classification of symptoms is valid today. The disease may follow any of four patterns: (1) that of acute intra-abdominal disease, resembling most frequently acute appendicitis; (2) that of ileocolitic diarrheal disease; (3) that of chronic intestinal obstruction with supervening acute obstruction; (4) that of fistula (external or internal) formation. Differential diagnosis demands consideration of acute appendicitis, bacillary dysentery, acute perforative peritonitis, intestinal obstruction and cancer of the bowel. Treatment is usually surgical. However the disease frequently recurs after surgical removal of the grossly diseased segments and doubtless heals in many instances without resection.

Holloway [308] presents 13 well studied and illustrated cases to show the sequence of the gross pathologic changes in the acute, subacute and chronic forms. The acute stage is characterized by pronounced edema attributed to lymphatic blockage and enlarged mesenteric nodes and accompanied with free intra-abdominal fluid. The subacute stage consists in infiltration, exudation and engorgement with a tendency to perforation and fistula formation. The chronic stage is manifested by stenosis, thickening and tumor formation. The acute stage may subside completely, and hence conservative treatment is recommended. In the chronic stage such resolution is unlikely; consequently short-circuiting procedures or resection are indicated. Owens [309] studied 22 cases and found neuromuscular hyperplasia to be a characteristic and hitherto unreported feature. Fallis,[310] in reviewing 32 cases, noted that in 40.7 per cent of them the patients had previously had appendectomies and that 30 per cent of the patients undergoing resection required subsequent further resection.

Wilensky [311] gives an interesting description of a woman in the fifth decade of life who had undergone an appendectomy twenty years earlier for "chronic appendicitis." Pain in the right lower quadrant of the abdomen recurred from time to time, becoming unusually aggravated in 1940. Roentgenologic examination showed the terminal segment of the ileum to be distorted and contracted for a distance of 5 inches (13 cm.). The patient recovered and remained well. The roentgenologic examination two years later showed no evidence of a lesion. Wilensky interprets this as a case of nonspecific granuloma of the terminal ileum with spontaneous healing.

Barnes [312] describes an instance in which the terminal ileum, the ascending colon and part of the transverse colon were involved. Resection of the terminal ileum and the right half of the colon has been performed for combined terminal ileitis and colitis.[313] An unusual and interesting case with the diagnosis of regional enteritis of the ascending colon is reported and discussed by Dr. Castleman, who emphasizes the significance of the presence of giant cells in the pathologic differention of this disease from chronic nonspecific ulcerative colitis.[314] The report of Phillips [315] is unusual because the patient was a 62 year old white man.

In a case of nonspecific enteritis involving Meckel's diverticulum, a fistula had developed after a previous appendectomy and right salpingo-oophorectomy.[316] A perforation was found in the diverticulum. Regional ileitis with concomitant ureteritis due to extrinsic pressure of the mass on the ureter is described.[317] After removal of the focus of infection and obstruction in the intestines, the ureter returned to normal.

In a 23 year old white man with extensive granulomatous jejunoileitis, a pronounced hypoproteinemia which responded only moderately to the injection of proteins and amino acids was observed.[318] A deficiency in pancreatic amylase complicated the disease. The oral administra-

308. Holloway, J. W.: Regional Ileitis, Ann. Surg. **118**:329-342, 1943.

309. Owens, F. M., Jr.: Regional Enteritis: Pathological Study of Twenty-Two Cases, Arch. Surg. **48**: 465-471 (June) 1944.

310. Fallis, L. S.: Regional Enteritis: Case Reports, Am. J. Surg. **62**:225-230, 1943.

311. Wilensky, A. O.: Spontaneous Healing in Nonspecific Granuloma of the Terminal Ileum, Rev. Gastroenterol. **11**:108-110, 1944.

312. Barnes, J. P.: Nonspecific Granuloma of the Colon, Texas State J. Med. **39**:529-532, 1944.

313. River, L. P.; Howser, J. W., and Vaughn, A. M.: Regional Ileitis: Management of Combined Involvement of Distal Ileum and Distal Colon, Am. J. Surg. **63**:118-123, 1944.

314. Case Records of the Massachusetts General Hospital, Cabot Case 30172, New England. J. Med. **230**: 526-529, 1944.

315. Phillips, J. R.: Regional Enteritis, Am. J. Digest. Dis. **11**:197-198, 1944.

316. Horn, R. C., Jr., and Rhoads, J. E.: Regional Enteritis Involving Meckel's Diverticulum, Ann. Surg. **119**:274-278, 1944.

317. Hyams, J. A.; Weinberg, S. R., and Alley, J. L.: Chronic Ileitis with Concomitant Ureteritis: Case Report, Am. J. Surg. **61**:117-120, 1943.

318. Killian, S. T., and Ingelfinger, F. J.: Nutritional Problems Presented by a Patient with Extensive Jejunoileitis, Arch. Int. Med. **73**:466-471 (June) 1944.

tion of a concentrated amylase was followed by a gain in weight, an increase in serum proteins and an apparent inhibition of the growth of Monilia albicans in the stools.

Cyst.—Rosenburg [319] reports on an enterogenous cyst of the ileocecal junction. The diagnosis may be suggested preoperatively by a palpable mass in the right lower abdominal quadrant. Pain from distention within the cyst is the most frequent symptom, but intestinal obstruction due to local pressure of the tumor and bleeding from an erosion of a blood vessel may occur. Marsupialization is recommended.

Timoney [320] resected successfully a mucocele of the appendix with local implantation of pseudomyxoma peritonei.

Benign Tumors.—Commenting on the rarity of benign neoplasms of the small intestine, Collins [321] analyzes 18 cases of neurofibroma collected from the literature and reports the case of a Negro man, aged 65, with symptoms of acute intestinal obstruction of forty-eight hours' duration and a history of a mass in his abdomen for one year. At operation a tumor the size of a grapefruit and with a twisted pedicle was found. Schottenfeld [322] presents a complete discussion of lipomas of the small intestine and ileum, including a review of the literature and report of 6 cases.

Gordon-Taylor [323] reports on a myoma of the midintestine resected successfully and also the case of a 67 year old man who had unexplained recurrent high intestinal bleeding, with no abnormality found at operation. However, at the autopsy, performed a few days later, a tumor the size of a small orange was found in the middle of the jejunum. The tumor, ulcerated on its mucosal surface, was histologically a leiomyoma. In 1 of 2 cases recurrent melena was due to a leiomyoma of the terminal portion of the jejunum and in the second to a degenerating leiomyosarcoma of the midportion of the ileum.[324]

Golden [325] reports that the ileocecal valve is rarely large enough to produce a recognizable indentation on the shadow of the barium-filled cecum, although it can sometimes be demonstrated on pressure films. Two cases are described in which the lips of the valve were enlarged. At operation hypertrophy of the lips of the valve with edema of the mucosa was found, the edema involving the terminal ileum and attributed to alcoholism with nutritional deficiency. In the second case the hypertrophy was also due to edema of the ileal side of the valve projecting into the cecum and producing early regional enteritis.

Carcinoid Tumors.—In a study of 130 carcinomas of the small intestine 30 so-called carcinoid tumors were found.[326] Thirteen of these had metastasized locally or distantly. Nine of the tumors were responsible for disabling symptoms, chiefly chronic intestinal obstruction. The lesions may occur anywhere from the esophagus to the anus, but they tend to be present in the terminal segments of the ileum as small orange submucosal nodules with minimal ulceration. Fifty per cent of the tumors were multicentric. Puckering and kinking were observed in the region of the tumor. Involvement of regional lymph nodes was observed in 11 and hepatic metastasis in 5. The term "little carcinoma" is apt, for large lesions are rarely encountered, although the metastatic deposits may be very large. Evidence is afforded that all carcinoid tumors are in essence peculiar low grade (1, Broders) adenocarcinomas. Nevertheless, it is suggested that the word "carcinoid" be appended by virtue of usage and as a designation of the peculiar mode of origin, life history and spread of these rather unusual neoplasms. They are always malignant, and metastasis occurs in at least a third.[327] Two cases of carcinoid tumors of the ileum are reported,[328] in both of which the symptoms of intestinal obstruction were present together with metastases to the regional lymph nodes. The unusual features in

319. Rosenburg, S. A.: Enterogenous Cysts at the Ileocecal Junction, Ann. Surg. **119**:873-877, 1944.

320. Timoney, F. X.: Ruptured Mucocele of the Appendix with Pseudomyxoma Peritonei, Am. J. Surg. **64**:417-419, 1944.

321. Collins, J. D.: Neurofibroma of the Small Intestine: Report of Case, Ann. Surg. **119**:362-371, 1944.

322. Schottenfeld, L. E.: Lipomas of the Gastrointestinal Tract with Special Reference to the Small Intestine Including the Ileum: Review of the Literature and Report of Six Cases, Surgery **14**:47-72, 1943.

323. Gordon-Taylor, G.: On Severe Intestinal Haemorrhage Due to Myomatous Tumour of the Jejunum with a Note on Bleeding Myomata of the Small Intestine, Brit. J. Surg. **31**:266-269, 1944.

324. Smith, L. A.; Good, C. A., and Gray, H. K.: Tumor of the Small Intestine as a Cause of Recurrent Melena, Proc. Staff Meet., Mayo Clin. **19**:117-122, 1944.

325. Golden, R.: Enlargement of the Ileocecal Valve, Am. J. Roentgenol. **50**:19-23, 1944.

326. Dockerty, M. B., and Ashburn, F. S.: Carcinoid Tumors (So-Called) of the Ileum, Arch. Surg. **47**:221-246 (Sept.) 1943.

327. Dockerty, M. B.; Ashburn, F. S., and Waugh, J. M.: Metastasizing Carcinoids of the Ileum, Proc. Staff Meet., Mayo Clin. **19**:228-235, 1944.

328. McLeod, C. E.: Carcinoid Tumors of the Ileum (Argentaffinomas), Am. J. Clin. Path. **14**:301-303, 1944.

the case of Korkosz [329] are a spontaneous perforation of the ileum proximal to the site of the lesion and a general atrophy of the colonic mucosa associated with a submucous fatty replacement. A clinical diagnosis of terminal ileitis was corrected by microscopic examination to argentaffinoma of the ileocecal valve.[330]

A carcinoid tumor of the cecum with local and distant metastases is reported [331] in a 70 year old man, the fifth case of carcinoid tumor of the cecum in the literature. .

Carcinoma.—Boman,[332] in a paper on primary carcinoma of the jejunum and ileum, reviews the literature briefly and reports 7 interesting cases. Metastases to the mesenteric nodes were present in all and to the omentum and peritoneum in 1. Black [333] reports ileoileocolic intussusception caused by a polypoid carcinoma of the ileum 40 cm. proximal to the ileocecal valve. In 16,318 necropsies performed at the Cook County Hospital, Chicago, there were only 3 cases of primary carcinoma of the jejunum, including 1 intussusception.[334]

Sarcoma.—A successfully resected sarcoma of the jejunum in association with von Recklinghausen's disease is described.[335] Though neurosarcoma occurs in 20 per cent of the cases of von Recklinghausen's disease, the occurrence in the jejunum is extremely rare. A malignant lymphoma with perforation of the small intestine is reported.[336]

APPENDIX

Acute Appendicitis.—Etiology: In 61 cases of catarrhal appendicitis, Bowen [337] found a stercolith in only 5 per cent, whereas in 63 cases with gangrene it was present in 63 per cent; from this he concludes that in the severe grades of appendicitis the stercolith is clearly an important factor.

Diagnosis: Tripodi and Kruger [338] report a case of appendical calculus recognized preoperatively by the presence of calcification in the right lower abdominal quadrant roentgenologically.

Shannon [339] is of the opinion that obstructive appendicitis should be suspected in children who complain of repeated gastric upsets with abdominal pain, nausea and vomiting and that appendectomy should be performed before acute appendicitis develops. Globe [340] distinguishes the generalized pain due to distention of the appendix from the localized pain due to irritation of the peritoneum. Hutchinson [341] reports bleeding from the inferior epigastric vessels in a 28 year old man with no history of trauma, in whom the symptoms suggested appendicitis. Myers and Rominger [342] report a retroperitoneal, retrocecal mucocele of the appendix of enormous size in a 72 year old patient. Newerla and Connally [343] report a case of gangrenous appendicitis in a strangulated femoral hernia of the Richter type. River and Gradinger [344] report appendiculocolic fistula with recurring attacks of pain in the right lower quadrant of the abdomen and a roentgenologic deformity of the cecum suggestive of neoplasm.

Hatchette [345] describes left-sided appendicitis diagnosed entirely by roentgenologic examination, and confirmed by operation and pathologic examination. The incomplete rotation of the colon was not discovered until the roentgenologic examination. The pain was located in the left side of the abdomen, just above the level of the anterior iliac spine. On roentgenoscopic examination the proximal half of the transverse colon and a portion of the ascending colon were parallel to the distal half of the transverse colon and the

329. Korkosz: Malignant Argentaffinoma of the Ileocecal Valve, Gastroenterology **1**:961-964, 1943.

330. Case Records of the Massachusetts General Hospital, Cabot Case 30241, New England J. Med. **230**:739-742, 1944.

331. Potter, E. B., and Docter, J. M.: Carcinoid Tumor of the Cecum, Am. J. Path. **20**:143-147, 1944.

332. Boman, P. G.: Primary Carcinoma of the Jejunum and the Ileum, Ann. Int. Med. **20**:779-788, 1944.

333. Black, B. M.: Polypoid Carcinoma of the Ileum Producing Intussusception: Primary Resection with Recovery of Patient, Proc. Staff Meet., Mayo Clin. **19**: 142-146, 1944.

334. O'Donoghue, J. B.; Lichtenstein, M. E., and Jacobs, M. B.: Primary Adenocarcinoma of the Jejunum with Intussusception: Case Report, Am. J. Surg. **63**:382-387, 1944.

335. Hamilton, J. B.; Kennedy, P. C., and Herault, P. C.: Neurogenic Sarcoma of the Jejunum Associated with von Recklinghausen's Disease, Ann. Surg. **119**: 856-864, 1944.

336. Case Records at the Massachusetts General Hospital, Cabot Case 30011, New England J. Med. **230**:20-24, 1944.

337. Bowen, W. H.: The Aetiology of Appendicitis, Brit. J. Surg. **31**:127-135, 1943.

338. Tripodi, A. M., and Kruger, A. L.: Appendiceal Lithiasis, Am. J. Surg. **61**:138-142, 1943.

339. Shannon, W. R.: The Crippled Appendix: A Pediatric Problem, Minnesota Med. **27**:466-468, 1944.

340. Globe, R. A.: Pain as a Symptom of Appendicitis, Minnesota Med. **26**:622-624, 1943.

341. Hutchinson, W. B.: Rectus Hemorrhage Simulating Appendicitis, Northwest Med. **42**:16-17, 1944.

342. Myers, W. H., and Rominger, R. F.: Retroperitoneal Mucocele of the Appendix: Case Report, Am. J. Surg. **63**:362-367, 1944.

343. Newerla, G. J., and Connally, E. F.: Gangrenous Appendicitis in Femoral Hernia of Richter's Type, Am. J. Surg. **61**:154-156, 1943.

344. River, L. P., and Gradinger, B. C.: Appendiculocolic Fistula: Report of a Case, Am. J. Surg. **61**:297-299, 1943.

345. Hatchette, S.: Roentgenologic Diagnosis of Left-Sided Appendicitis: Report of a Case, Am. J. Roentgenol. **50**:244-247, 1943.

cecum lay parallel to the descending colon and anterior to it. The appendix filled well, was painful to pressure and contained a moderate-sized filling defect at the tip. At operation the tip of the appendix was included in an inflammatory process forming an abscess and involving also the mesentery of the small bowel of the left side. Since most reports in the literature state that the pain in left-sided appendicitis occurs on the right side, the location of the pain in this instance is ascribed to involvement of the mesentery. Prescott and Zollinger [346] report 3 cases of acute appendicitis in situ inversus totale, in none of which the symptoms were referable to the right side.

Fraser,[347] in a well documented historical, clinical, etiologic and pathologic study of intussusception of the appendix describes 7 instances of simple intussusception of the partial and complete types and refers to 82 cases in the literature. The characteristic clinical feature is the occurrence of very severe attacks of colic with periods of complete remission. Complete inversion of the appendix into the cecum may be associated with cecocolic intussusception and the appendix palpated at the rectum.[348]

Treatment.—Behrend [349] in reviewing 4,283 cases found that in 2,459 cases of acute appendicitis the mortality was 1.34 per cent. Apparently the mortality from appendicitis has been kept at a very low level in the last decade chiefly because of prompt diagnosis and operation and also because the number of interval and prophylactic appendectomies has reduced the number of possible cases [350] of acute disease.

Spencer [351] reports 100 cases of suppurative and gangrenous appendicitis in patients treated in a small rural hospital with a mortality of 2 per cent. In 25 cases with perforation the mortality rate was 4 per cent. Chenoweth [352] reviews a series of 149 cases of appendical abscess. In 60 per cent the patients were subjected to appendectomy with drainage and in 40 per cent to incision and drainage only. The general mortality was 4.7 per cent. Of the patients whose appendixes were not removed either at the time of drainage or at a secondary elective operation 40 per cent had recurrent abscess.

In a review of 903 cases of perforative appendicitis both the morbidity and the mortality were reduced by the use of sulfonamide drugs, the mortality rate decreasing in the years from 1939 to 1942 from 9.2 per cent to 3.4 per cent as the use of sulfonamide compounds increased.[353]

DYSENTERY AND DIARRHEAL DISEASES

Epidemic Diarrhea of the Newborn.—Geiger and Sappington [354] report on an epidemic of diarrhea in the newborn infants, 324 cases with 45 deaths. Most of the patients were exposed apparently to infection during the first two weeks of life, and enteritis developed one to two weeks later. No definite etiologic factor was identified. Sakula [355] studied an outbreak of gastroenteritis in the nursery of a maternity department in which 18 infants contracted the disease and 15 died, a mortality of over 80 per cent. The dissemination was confined to bottle-fed infants. The observations at necropsy suggested an acute toxic condition rather than an intestinal infection. Pseudomonas pyocyanea was isolated from the stools or elsewhere in 11 of the 25 cases.

Draper [356] reports 22 cases of gastroenteritis in children under 5 years of age, Bacillus typhimurium being isolated from the stools in 20 and Bacillus Reading in 2.

Light and Hodes [357] studied six separate epidemics occurring in three hospitals in the Baltimore-Washington area from the standpoint of a possible filtrable agent. In four of the epidemics a filtrable agent was isolated which regularly produced diarrhea in calves. This agent was not recovered from the stools of nor-

346. Prescott, M. U., and Zollinger, R. W.: Appendicitis in Situs Inversus Totalis, Am. J. Surg. **64**:288-290, 1944.

347. Fraser, K.: Intussusception of the Appendix, Brit. J. Surg. **31**:23-33, 1943.

348. McCrea, A. N., and Gans, B.: A Case of Intussusception of the Appendix, Brit. J. Surg. **31**:197-198, 1943.

349. Behrend, M.: Appendicitis: A Review of 4,283 Cases, Am. J. Surg. **63**:90-95, 1944.

350. Boone, H. R., and Lyons, S. C.: The Mortality of Acute Appendicitis: Its Reduction by Diagnostic and Therapeutic Methods, U. S. Nav. M. Bull. **41**:1273-1283, 1943.

351. Spencer, J. H., Jr.: Management of Acute Suppurative Appendicitis in the Small Rural Hospital: A Report of One Hundred Consecutive Cases, Am. J. Surg. **61**:249-258, 1944.

352. Chenoweth, A. I.: Appendiceal Abscess, Surgery **14**:702-707, 1943.

353. Stafford, C. E.; Beswick, J., and Deeb, P. H.: Evaluation of Sulfonamides in the Treatment of Peritonitis of Appendiceal Origin: A Review of 903 Cases of Acute Perforative Appendicitis, Am. J. Surg. **64**:227-234, 1944.

354. Geiger, J. C., and Sappington, E. E.: Epidemic Diarrhea of the Newborn in San Francisco—1943, Arch. Pediat. **61**:134-145, 1944.

355. Sakula, J.: An Outbreak of Gastro-Enteritis in the Newborn, Lancet **2**:758-760, 1943.

356. Draper, F.: Gastroenteritis in Children: A Report on the Bacteriology of Twenty Cases Caused by Bacterium Typhi-Murium and Two Cases Caused by Bacterium Reading, M. J. Australia **1**:533-539, 1944.

357. Light, J. S., and Hodes, H. L.: Studies on Epidemic Diarrhea of Newborn: Isolation of a Filtrable Agent Causing Diarrhea in Calves, Am. J. Pub. Health **33**:1451-1454, 1943.

mal infants or normal calves. The evidence suggested, therefore, that the agent might be a cause of epidemic diarrhea of the newborn. Cultures of stool were uniformly negative for known diarrhea-producing organisms. The filtered stools were given by nasal inoculation. Successive passages through calves were found readily possible with each of the four strains, and the results of studies of cross immunity indicated that the four strains represented a single agent. The disease was produced in a total of 84 calves. Reimann, Price and Hodges [358] studied an epidemic of diarrhea, nausea and vomiting of unknown cause, excluded food poisoning, found none of the usual bacteria or other parasites commonly associated with enteritis and attempted to isolate a filtrable infectious agent similar to that described by Light and Hodes, with negative results in the 8 experiments performed with fecal material preserved frozen with solid carbon dioxide for two months. Gunn [359] reviews 411 cases of acute gastroenteritis. Glaser and Bruce [360] report that in treatment the administration of unlimited amounts of butter milk, skimmed milk and protein milk decreased the period of hospitalization and the death rate.

Vitamin Deficiency.—Wintrobe and his associates [361] found that pantothenic acid deficiency in young pigs was characterized by diarrhea, then dysentery and loss of appetite, striking impairment of growth, loss of hair, cough and excessive nasal secretion, changes in the tongue and abnormal gait. Extensive colitis was shown by hyperemia, edema and ready bleeding of the bowel when examined rectosigmoidoscopically. At autopsy the changes ranged from diffuse hyperemia with increase in the size of the lymphoid follicles and the formation of small ulcers to extensive inflammatory changes involving the entire intestine. Histologically, atrophy of the cells lining the glands of the mucosa, abscess formation and ulceration were observed. The administration of 500 or more micrograms of calcium pantothenate per kilogram of body weight per day was accompanied with cessation of diarrhea, gradual improvement in the condition of the bowel, restoration of normal blood values, growth of hair and gain in weight.

Food Poisoning.—Tapp [362] in reviewing the causes of food poisoning lists the following: bacterial causes as Salmonella, staphylococcus toxin and Clostridium botulinum; "milk sickness" due to trematol, a poisonous substance contained in weeds eaten by cattle and excreted in their milk; fungus poisoning, from inedible mushrooms; ergotism, from grain; mussel poisoning, and cadmium poisoning, from the metal used to plate cooking utensils. Hoechstetter [363] discusses shellfish and mushroom poisoning. DeLay [364] describes an outbreak of characteristic staphylococcic food poisoning in which enterotoxigenic staphylococci were obtained from a bread pudding served at the meal preceding the outbreak. About 400 of the 600 men served were affected. The pudding was allowed to stand at room temperature (75+ F.) for over twenty-four hours. The outbreak illustrates the need for adequate facilities for refrigeration and their use.

Bacillary Dysentery.—The most important of the diarrheal diseases, according to Callender [365] is bacillary dysentery. Typhoid and the dysenteries ordinarily increase under the same unsanitary conditions. In the present war, however, typhoid vaccine appears to have controlled the incidence of this disease, while bacillary dysentery, for which no immunization has been developed, has risen in troops to heights equal to those of twenty years ago.

Hardy and Watt [366] report on an excellent study carried out in Puerto Rico, New Mexico, Georgia and New York city. Satisfactory clinical data were obtained in 1,247 of 1,499 cases, with an epidemiologic history of 830 households. The term "shigellosis" is suggested for all infections due to pathogenic varieties of Shigella.

During the months of May, June and July 1943 an outbreak of acute enteritis occurred

358. Reimann, H. A.; Price, A. H., and Hodges, J. H.: Negative Results in Studies of Epidemic Diarrhea, Nausea and Vomiting of Unknown Cause, Proc. Soc. Exper. Biol. & Med. **55**:233-234, 1944.

359: Gunn, W.: Acute Gastroenteritis in Children: A Review of 411 Cases, 1936-1943, and a Plan of Investigation and Treatment, Brit. J. Child. Dis. **41**:1-10, 1944.

360. Glaser, K., and Bruce, J. W.: Treatment of Epidemic Diarrhea and Dysentery in Infants and Young Children, J. Pediat. **24**:53-61, 1944.

361. Wintrobe, M. M.; Follis, R. H., Jr.; Alcayaga, R.; Paulson, M., and Humphreys, S.: Pantothenic Acid Deficiency in Swine, with Particular Reference to the Effects on Growth and on the Alimentary Tract, Bull. Johns Hopkins Hosp. **73**:313-333, 1943.

362. Tapp, E. M.: Food Poisoning, M. Bull. Vet. Admin. **20**:61-66, 1943.

363. Hoechstetter, S. S.: Food Poisoning, M. Bull. Vet. Admin. **20**:58-60, 1943.

364. DeLay, P. D.: Staphylococcal Enterotoxin in Bread Pudding, Bull. U. S. Army M. Dept., 1944, no. 72, pp. 71-73.

365. Callender, G. R.: Dysenteries and Diarrheas: Their Importance in Military Service, War Med. **4**:459-464 (Nov.) 1943.

366. Hardy, A. V., and Watt, J.: (*a*) The Acute Diarrheal Diseases, J. A. M. A. **124**:1173-1179 (April 22) 1944; (*b*) Newer Procedures in Laboratory Diagnosis and Therapy in the Control of Bacillary Dysentery, Am. J. Pub. Health **34**:503-509, 1944.

among Army personnel stationed in North Africa. It was characterized by two types of enteritis, one mild and the other severe. The causative organisms were Shigella paradysenteriae (Flexner) 73 per cent, Shigella ambigua 11 per cent, unidentified paradysentery bacilli 9 per cent, Shigella sonnei 3 per cent, Shigella madampensis 2 per cent and Shigella enteritidis 2 per cent. The wide distribution of the Shigella paradysenteriae (Flexner) was proved by the fact that it was identified in other laboratories in other areas in North Africa. Gowen,[367] on epidemiologic grounds, concludes that transmission by flies was probably the most important factor in the spread of the disease, although contact with carriers and persons with mild unrecognized forms of the disease undoubtedly played an important part once the troops had been seeded with the infection. Adams and Atwood,[368] in a study of 251 cases of bacillary dysentery found shigella organisms in 90 per cent of the patients, salmonella organisms in 6 patients and an organism belonging to the paracolon group in 33 patients. Of the shigella organisms found, 70.9 per cent were Shigella paradysenteriae and 8 per cent Shigella Newcastle. These responded to therapy with sulfonamide compounds. Twenty-eight infections due to Shigella sonnei and Shigella alkalescens did not respond to chemotherapy.

Bacillary dysentery occurs in Chicago as a sporadic disease throughout the year, with greater frequency in late summer and autumn months, 307 cases having been encountered at the Cook County Hospital during the years 1938 to 1941 inclusive.[369] Some patients acting as "carriers" harbor the organisms over extended periods, in this series as long as seventy days. The mortality during the years 1938 to 1941 inclusive was 7 per cent, the majority of deaths occurring among the very old and the very young. Felsenfeld and Young,[370] in 13,000 stool examinations of the state hospitals of the greater Chicago area, isolated 64 strains of Salmonella belonging to 19 types. Organisms were frequently found in symptomless carriers. Treatment of the carriers with sulfonamide compounds resulted in failure. A number of dysentery infections were successfully treated with sulfadiazine and sulfathiazole; but those due to Salmonella typhimurium, the most common type, were resistant. A new three sugar medium has been devised[371] for the differentiation of Salmonella and paracolon organisms. Littman[372] describes a rapid method for the isolation and identification of the enteric pathogenic organisms from the feces, utilizing standard mediums and generic charts of fermentative reactions and serologic characteristics. Larkum[373] discusses the bacteriologic classification. Wheeler[374] describes a method for the serologic identification of the pathogenic dysentery bacilli, utilizing a slide agglutination test, and presents the result of the serologic typing of 1,005 Shigella strains.

Acute dysentery in an infant 14 months of age is attributed to Shigella alkalescens.[375] The pathologic changes were located primarily in the lymphoid tissue in the colon and in the ileum and contrasted with the diffuse involvement of the mucosa when Shiga and Flexner organisms are present. Attempts to infect animals were unsuccessful, although Edward did produce lesions in the rabbit almost identical to those seen in this case. Stuart and co-workers[376] isolated from the stools of sick infants, normal exposed infants and normal adults having contact with the infants during an epidemic of gastroenteritis Shigella alkalescens and antigenically related organisms, forming an almost perfect intergrading series biochemically from S. alkalescens to Escherichia coli, thus suggesting their probably evolutionary development.

Seligmann and Hertz[377] isolated fifteen different types of Salmonella in 37 cases observed at the Beth Israel Hospital. One or more types from each of the Salmonella groups was isolated

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in Florida.[378] A new Salmonella type obtained from the stool specimens of a normal food handler has been designated Salmonella mississippi.[379]

Ferguson and Hook [380] describe a method of distinguishing proteus and salmonella organisms by their growth on a medium containing urea, the proteus organisms decomposing the urea and the salmonella organisms showing no activity.

Snapper,[381] in an analysis of the acute outbreaks of salmonella infections in Western Europe and also in the United States attributed to contaminated duck eggs, shows that the disease usually does not result directly from the ingestion of infected eggs, the number of organisms in any one egg being small, but rather is due to the infection of custards, puddings and other dishes in which growth and multiplication of the organisms occurs.

Coughlin [382] describes a hotel outbreak of gastroenteritis affecting 83 persons, including 70 of the 78 persons attending a college alumni dinner, 10 of the 19 attending an insurance company's dinner, 2 members of the hotel staff and a guest at the hotel. The infection was attributed to chef's salad prepared by the assistant chef, who was the only hotel employee who remained well and in whose stools Salmonella derby were found. The evidence, however, was not conclusive. Coleman, in discussing this paper, points out that more than 100 species of Salmonella are now recognized and suggests that while human carriers must be recognized as a potential source of infection, even though epidemiologic studies seldom indicate transmission from person to person, the frequency with which various salmonella organisms have been found in tissues from apparently normal as well as diseased animals and birds suggests that they constitute the main reservoir for human infection.

An outbreak of milk-borne gastroenteritis due to Salmonella dublin affected 162 persons.[383] The source of the infection was traced to an apparently healthy cow excreting large numbers of the organisms. An epidemic of sonne dysentery was due apparently to an infected water supply, as the bacillus was isolated from a sample of the water which had passed satisfactorily the usual bacteriologic tests after chlorination.[384] Hailwood [385] describes an outbreak of Sonne dysentery spread by food infected by a chronic carrier who gave a history of diarrhea one year previously. He was the only person whose stools contained Bacillus dysenteriae Sonne who was asymptomatic during the period of the epidemic. Zellweger,[386] in a study of an epidemic of bacillary dysentery (type Sonne-Kruse) occurring in soldiers in the field and attributed to the contamination of the food by flies, emphasizes the importance of prophylaxis because of the enormous disability occasioned by the epidemic.

Ethylene glycol and phenol extracts of the Shiga bacillus produce considerable immunity in the mouse to infection with fully toxigenic strains of Bacterium shigae.[387] Large doses of formaldehyde-treated vaccine induce resistance to infection in mice almost as well as the extract but fail to elicit agglutinins to the same extent. In an effort to find an agent which would be polyvalent and relatively nontoxic for the prophylactic immunization of human beings against dysentery bacilli infections, an antigen prepared from type V Shigella paradysenteriae was found to give rise in human beings to antibodies which are broadly cross reactive.[388] The toxic properties of the antigen are undesirable but apparently not prohibitive when the antigen is used in the small quantities required. Whether it will afford protection against bacillary dysentery awaits trial in the field. Age apparently is not

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a significant factor in susceptibility to the endotoxin of S. paradysenteriae.[389]

D'Antoni[390] distinguishes chronic bacillary dysentery in which there is a continuation of the dysenteric stools, with pus and blood, from Shigella colitis, in which there is only a diarrhea of liquid feces. In the latter the organisms may be difficult to demonstrate, 20 examinations of stools being required in some instances. In the 150 cases observed an average of 5 stool examinations were required before a positive diagnosis was made.

Felsenfeld,[391] with special reference to the Monilia-Cryptococcus-Geotrichum group of fungi, investigated the mycotic flora of the intestinal tract of institutionalized patients in an effort to find possible causes of enteric infections other than the common pathogenic bacteria and parasites. The first group of patients consisted of 300 newly admitted persons and 600 from the wards for chronic disease, all without intestinal disturbances. The second group consisted of 100 chronic typhoid carriers. The third group, composed of patients with diarrhea, was subdivided into these classifications: 47 in the early acute stage of Shigella sonnei and paradysentery infections; 51 with bacillary dysentery one week after termination of treatment with sulfathiazole, and 103 who had food upsets with diarrhea of short duration and without determinable cause. Of the newly admitted patients 26.6 per cent had no fungi. The three species of fungi were found in about equal numbers in the remainder, and in the majority only one species was present. In the patients from the chronic ward and the typhoid carriers there was some tendency to harbor two or three species. In the "food upset" group the incidence of Geotrichum doubled with a 50 per cent increase in the number harboring two species. In the group with bacillary dysentery the incidence of Monilia organisms increased. In the patients with dysentery treated with sulfathiazole there was an absolute and a relative decrease in the incidence of all three species of fungi.

Chemotherapy.—Succinylsulfathiazole was given to 3 dysentery carriers in amounts of 0.147, 0.195 and 0.220 Gm. per kilogram of body weight daily.[392] These amounts did not successfully eliminate the organisms from the stools when given for seven days, but when given for fourteen days they did so. A fourth patient did not respond to 0.166 Gm. of the drug per kilogram daily for fourteen days but was rendered free from dysentery bacilli by 0.25 Gm. per kilogram daily for five days. A fifth patient recovered spontaneously from the carrier state. Although relatively small amounts of succinylsulfathiazole are effective if continued for a long time, a dosage of 0.25 Gm. per kilogram daily for five to seven days should uniformly eliminate dysentery bacilli from the stools of carriers in a short time.

In an outbreak of Sonne dysentery involving 50 children in an orphanage with a total census of 145, organisms were found in the stools of 83.[393] The administration of sulfathiazole and sulfadiazine in doses of 1 grain (0.06 Gm.) and sulfaguanidine and succinylsulfathiazole in doses of 2 grains (0.13 Gm.) per pound (0.5 Kg.) of body weight cleared 90 per cent of the positive stools in four days. The remaining 10 per cent were cleared with one or two additional courses of treatment. The administration of the drugs caused complete inhibition of growth of intestinal bacteria for a time in 80 per cent of the persons treated with sulfadiazine, 63 per cent of those treated with succinylsulfathiazole and 37 per cent of all treated with sulfaguanidine.

Sulfaguanidine has been used during the early convalescent stage of Sonne bacillary dysentery in an attempt to shorten the carrier state; the attempt was unsuccessful when one fourth of the therapeutic dose was given daily, but in another experiment in prophylaxis 2 Gm. daily for ten days given to thirty-six persons at the outset of an epidemic apparently prevented dysentery, whereas of 45 persons used as controls 9 contracted the disease.[394] Smith[395] treated 44 young women, mainly symptomless carriers of Bacillus dysenteriae (Flexner), with massive doses; the bacteriologic results were satisfactory, but in 21 of them a toxic rash developed on about the ninth day of treatment. Eight of 12 patients

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reacted to a sensitization dose of other sulfonamide compounds; this fact suggests that the guanidine radical may be the sensitizing agent.

Thirty carriers of Shigella paradysenteriae were treated with 20 Gm. of sulfaguanidine for six days and 55 with 20 Gm. of succinylsulfathiazole for six days.[396] Dysentery bacilli disappeared from the stools in every patient, the criterion being a minimum of nine consecutive negative reports after cessation of treatment. No significant toxic reactions attended the use of either drug, but the patients receiving succinylsulfathiazole experienced minor annoyances. Page [397] reports on 208 cases of bacillary dysentery treated with sulfaguanidine with no deaths or serious complications; in 91 per cent Shigella paradysenteriae was found and in 8 per cent Shigella sonnei. In 2.8 per cent of the cases, in all of which the disease was due to S. paradysenteriae, negative stools were not obtained after a ten day course with sulfaguanidine; these responded to a five day course of succinylsulfathiazole. In a study of 200 cases of confirmed bacillary dysentery, sulfaguanidine gave results superior, both clinically and bacteriologically, to those from aperients and chalk, but, even so, 30 per cent of the patients still had bacteriologically positive stools in convalescence, as compared with 50 per cent with the other treatments.[398] Pyrexia did not appear to influence the effect of sulfaguanidine to any great extent. The use of broth enrichment technic revealed many positive results which would have been missed if direct plating had been employed. Baker [399] treated 26 patients with sulfaguanidine and 31 patients with sodium sulfate. The patients receiving sulfaguanidine had a more rapid reduction in the diarrhea, less toxemia and a quicker return of the rectal mucosa to normal on proctoscopic examination. Chronic Flexner dysentery treated successfully with succinylsulfathiazole is reported.[400]

Poth and Ross [401] found that when N4-phthalylsulfathiazole is fed to dogs in doses of 0.25 Gm. per kilogram of body weight per day the coliform organisms may be completely eliminated from the bowel in three days. There was no evidence of toxicity. The bacteriostatic effect is two to four times that of succinylsulfathiazole. With this dosage the concentration of the drug in the blood did not exceed 3 mg. per hundred cubic centimeters. Callomon and Raiziss,[402] studying the bactericidal action of succinylsulfapyrazine and malylsulfathiazole against the intestinal coliform flora of normal mice, found the former to be more effective than succinylsulfathiazole and equal to sulfaguanidine, whereas the latter approximated succinylsulfathiazole in effectiveness. Four new sulfonamide drugs — sulfamerazine, phthalylsulfadiazine, phthalylsulfamerazine and phthalylsulfathiazole — were tested in vitro by Schweinburg and Yetwin for their action against certain enteric pathogens.[403] On the whole, the action of these drugs was similar to that of the older sulfonamide compounds. The shigellas proved more susceptible than other organisms.

Boyd and Portnay [404] found that bacteriophage of high specific potency was of no prophylactic or therapeutic value in treatment of bacillary dysentery.

Cholera.—Huang [405] reports that only 1 of 22 patients with Asiatic cholera treated with sulfaguanidine died and that the patients showed general improvement from three to four hours after the beginning of treatment.

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(To Be Concluded)